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Histogenetic and Organogenetic Processes in the Development of Specific Characters in some South African Tadpoles[1]

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INTRODUCTION

EVER since it was discovered that embryonic inductions can occur even where the inductor and the reacting tissues belong to different species of animals, it has been accepted that the specific nature of the reaction depends mainly on the properties of the reacting cells and not on the properties of the inductor. The embryonic cells appear to have only a limited set of possible reactions, determined by the genotype, whereas the inducing factors in different animals seem to be very similar and readily interchangeable. So the tissues of a species of salamander not normally possessing a balancer cannot develop one even if transplanted to another species which normally has that organ (Mangold, 1931; Rotmann, 1935*a*). Ectoderm of a frog embryo transplanted to the oral region of a salamander embryo will develop mouth parts, but these will be the mouth parts of a frog larva, with horny jaws and horny teeth, and not those of a salamander larva (Spemann & Schotté, 1932; Spemann, 1938). Rotmann (1939) has claimed that in the induction of the lens by the eye-cup the size of the induced lens is determined by the reacting epidermis. Although an undersized lens may be the result of a weaker stimulus, or of a stimulus confined to a very small area, the epidermis cannot react to an excessively large eye-cup by producing an abnormally large lens (even though a certain adjustment may be achieved later through accelerated growth of a lens which is relatively too small).

Striking as these results are, it may still be queried whether some distinctions in the structure of animals may not depend on different properties of the inducing systems. One case of this kind has already been established. It is known that the dorsal fin of amphibian larvae is induced by the neural crest (Terni, 1934; Du Shane, 1935). As the dorsal fin does not develop in the head region in spite of the presence of the neural crest, it follows that either the head epidermis is not

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competent to react to the inductor by forming a fin fold, or that the neural crest of the head is not capable of inducing a neural fold. By transplanting trunk neural crest cells under the epidermis of the head Terentiev (1941) proved the second alternative to be true: head epidermis developed a fin fold under the influence of the transplanted neural crest. As the cranial extension of the dorsal fin fold varies in different amphibians, it is probable that these distinctions may be referred to differences in the inducing system. In *Eurycea bislineata* the dorsal fin fold is absent in the whole of the trunk region and present on the tail only. Bytinski-Salz (1936) has proved that in this species the trunk epidermis is capable of producing the fin fold when transplanted to the tail. The absence of a fin fold on the trunk is thus the result of a failure of the trunk neural crest to act as an inductor.

In view of the possibility of the existence of two types of reactions determining the specific differences in the development of animals, it would be of interest to know what is the relative importance of each type of reaction in nearly related species. It is conceivable that while tissues of distantly related animals, such as *Urodela* and *Anura*, can only react in their own specific way, tissues of more nearly related forms might be capable of adjusting their reactions to fine differences in the inducing systems.

The embryos of several species of *Anura* found in Transvaal offer a most favourable material for such an investigation. Besides presenting forms of different degrees of affinity (from species of one genus to those of different families), the embryos and early larvae show a great variety of distinctions in the structure and size of their organs. By making heteroplastic transplantations I could thus test the specificity of the reactions in each case.

DISTINCTIONS IN THE DEVELOPMENT OF SPECIES USED

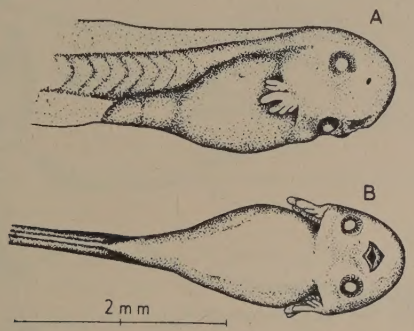
Four species were used: *Pyxicephalus delalandii* (Tschudi), *Pyxicephalus adspersus* (Tschudi), *Phrynobatrachus natalensis* Smith, and *Xenopus laevis* Daud. The first three belong to the family Ranidae, the fourth to the family Pipidae. The organs investigated were: the larval adhesive organs, the external gills, and the mouth parts with the oral disks, jaws, and teeth.

The normal development of all four species has been described previously: *P. adspersus* by Power (1927*b*, 1927*c*), *P. delalandii* by Hewitt & Power (1913) and by Power (1927*b*), *Phrynobatrachus natalensis* by Power (1927*a*). The development of *Xenopus* is well known from numerous papers, of which I need only mention the original description by Bles (1906) and the normal tables by Weisz (1945) and Faber (1955). Some of the features of early development, however, have not been dealt with at all, such as the adhesive organs and the external gills in the first three species. It will be useful therefore to review those features which have lent themselves to experimental analysis.

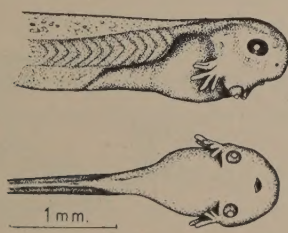
General size. Pyxicephalus adspersus has relatively very large eggs developing

into large embryos; two species, *Pyxicephalus delalandii* and *Xenopus laevis*, have middle-sized eggs and embryos, and the eggs and embryos of *Phrynobatrachus natalensis* are very minute. The diameters of the eggs are as follows: *Pyxicephalus adspersus*, 1·6–1·8 mm.; *Pyxicephalus delalandii*, 1·2–1·4 mm.; *Phrynobatrachus natalensis*, 0·5–0·6 mm.; *Xenopus laevis*, 1·1–1·2 mm. The relative size of the tadpoles can also be judged from Text-figs. 1–4, drawn to the same scale.

Adhesive organ ('*sucker*'). The initial stages of development of this structure in the ranid species are similar: the earliest rudiment is a somewhat thickened V-shaped area of epidermis with the cells assuming a columnar shape. This early stage is quickly passed in *P. delalandii* and in *Phrynobatrachus*, the middle part of the V disappearing and the antero-lateral ends of the rudiment developing



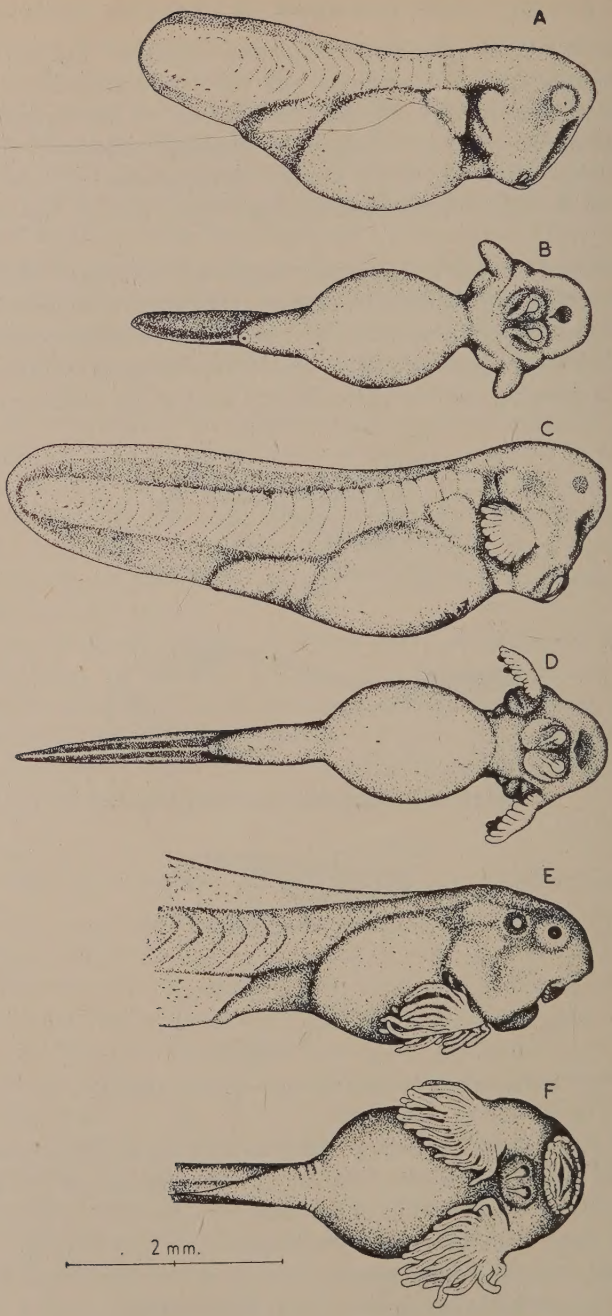
TEXT-FIG. 1. *Pyxicephalus delalandii*, stage 22.



TEXT-FIG. 2. *Phrynobatrachus natalensis*, stage 22.

into a pair of nipple-shaped conical projections with more or less obtuse summits, situated posterior to the corners of the mouth (Text-figs. 1 and 2). The adhesive organs of the two species are thus of the same type, but in *P. delalandii* they are considerably larger, conforming to the larger size of the embryo. In *Phrynobatrachus* the adhesive organs appear more pointed, due to the flattened tip being relatively smaller in proportion to the whole organ.

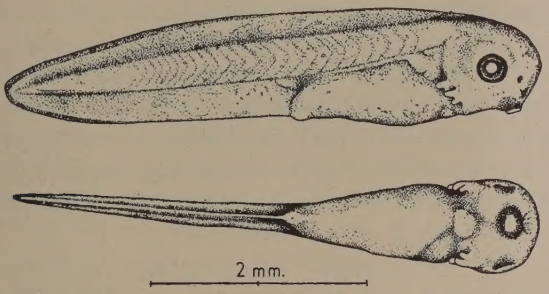
In *P. adspersus* the adhesive organ retains the V shape in its fully developed form. The anterior limbs of the V become somewhat rounded at the end, and a



TEXT-FIG. 3. *Pyxicephalus adspersus*. A, B: stage 18, external gills as spatulate processes. C, D: stage 20, beginning of development of gill filaments. E, F: stage 23, external gills fully developed.

thickened edge surrounds the glandular area on all sides, projecting in the form of a superficial languette between the limbs of the V anteriorly. The adhesive organ thus resembles that of a bufonid. The organ forms a very prominent swelling posterior to the mouth (see Text-fig. 3).

In *Xenopus* the rudiment of the adhesive organ is not V shaped but transversely oval. The cells of the rudiment later concentrate to form a single median adhesive organ, in the shape of a conical projection with a flattened top, lying just behind the mouth (Text-fig. 4).



TEXT-FIG. 4. *Xenopus laevis*, stage corresponding to stage 22 of the ranid embryos.

External gills. The external gills of *P. delalandii* and *Phrynobatrachus* are of the ordinary *Rana* type. There are two pairs of gills present, the anterior pair being larger and more ramified (having more gill filaments). Fully developed gills of *P. delalandii* have normally 4 gill filaments on the first gill and 3 on the second (Text-fig. 1). The gills of *Phrynobatrachus* have 3 or 4 filaments on the first gill and usually 2 on the second gill (Text-fig. 2). The gills of *P. delalandii* are much larger, longer, and thicker, than those of *Phrynobatrachus*, in conformity with the larger size of the tadpole. Further gill filaments start developing in both species whilst the opercular fold covers the external gills, thus converting them into internal ones.

The external gills of *P. adspersus* are most peculiar, both in respect of their structure when fully developed and in respect of their mode of formation. There is only one pair of external gills present: the first pair. In the stage when in other ranids the external gills make their first appearance as small tubercles (stage 19, Pollister & Moore, 1937), a very peculiar outgrowth is formed on the first branchial arch of a *P. adspersus* embryo. This is spatulate, flattened in the vertical plane and projecting from the body outwards and somewhat dorsad (Text-fig. 3 A, B). The outgrowth next broadens distally and bends backwards, still retaining the spatulate shape. The edge of the outgrowth now becomes cut up by deep clefts into lobes (Text-fig. 3 C, D). The clefts cut in deeper and deeper, and the strands of tissue between them become the filaments of the gill. The proximal part of the spatulate process is not dissected and remains intact serving

as a common base for all the filaments (Text-fig. 3 E, F). The number of the filaments is relatively very high: up to 16 on each gill. As the gill develops it gradually changes position, the plane of the gill which was originally vertical becomes almost horizontal, so that the filaments of the right and left gill form together a sort of double fan spread under the belly of the tadpole (Text-fig. 3F). This position of the fully developed gills is probably correlated with another curious peculiarity of the early tadoples of *P. adspersus*. As was first recorded by Power (1927*c*) and as I have observed myself, the newly hatched tadpoles of this species have a habit of swimming on their backs, upside down. When the tadpoles swim near the surface, as they usually do, the gill filaments are pressed against the water-air interface, so that they are in the best position for receiving oxygen. This may be of importance as *P. adspersus* breeds in shallow stagnant water in which an oxygen deficiency may easily develop.

Xenopus has two pairs of external gills. These are very poorly developed as compared with the gills of ranid tadpoles. The gills remain very short and the development of filaments is rudimentary, no more than two appearing on each gill, the second gill remaining often unbranched.

Mouth parts. The mouth parts of *Pyxicephalus adspersus*, *Pyxicephalus delalandii*, and *Phrynobatrachus natalensis* are described in the papers quoted above. They consist of a pair of jaws with sharp horny edges ('beak') surrounded by an oral disk bearing several rows of horny labial teeth anterior and posterior to the mouth. The edge of the oral disk is formed by a festoon of oral papillae. The tadpoles of *Xenopus*, being plankton feeders, have neither horny jaws nor labial teeth. They do not have an oral disk with oral papillae, and the mouth is very much smaller than in ranid tadpoles.

METHODS

The experiments consisted in removing parts of the presumptive ectoderm in the early gastrula stage and replacing them by pieces of presumptive ectoderm of an embryo of a different species. The grafted ectoderm was thus exposed to inductive influences of the deeper lying parts of the host and made to differentiate in conformity with these influences. As a rule the grafted tissue developed in accordance with its new position and fitted into the organization of the host embryo, preserving at the same time the peculiarities of the donor species in varying degrees.

The stages used for the operation were stages 9, 10, and 11 (Pollister & Moore, 1937). In most cases the graft was taken from the area at the animal pole which was as yet not underlain by endo-mesoderm. Occasionally some endo-mesodermal cells were already present at the edges of the area cut out for transplantation. These were then carefully removed. The site prepared for the graft was usually free of endo-mesoderm, but in some cases the graft came to lie over invaginated endo-mesoderm. Operations were performed in double-strength Holtfreter solution. This prevents the excised graft from curling and thus ensures

its smooth healing in the new position. The grafts were pressed down by pieces of coverslip for about half an hour, and the operated embryos were then transferred to tap-water. No attempt was made to orient the grafts in any specific way. It may thus be presumed that the organs developed in the graft were as a rule derived from cells having a different prospective significance.

The embryos were reared for several days; most of them were preserved in stages 22–23, with fully developed gills and adhesive organs. A smaller number were allowed to develop to stage 25, when the main features of the mouth armament may be discerned. Rearing the operated tadpoles through the feeding stage has so far not been successful. Sketches of the operated embryos were made at different stages and careful camera lucida drawings of all preserved tadpoles were prepared, after which all were embedded and sectioned.

The eggs for the operations were collected in pools in the suburbs of Johannesburg, except for some of the eggs of *Xenopus* which were obtained in the laboratory by injecting the frogs with prolane. As the spawning of the frogs was not under control, not all the heteroplastic combinations between the four species could be performed, but only the following:

P. adspersus ⇌ *P. delalandii*
P. delalandii ⇌ *Phrynobatrachus*
P. delalandii ⇌ *Xenopus*
Phrynobatrachus ⇌ *Xenopus*

The following analysis is based on 57 successful operations.

Tracing the grafts after the operation was comparatively easy. The four species differ in pigmentation: dense black in *P. adspersus*, brownish-grey in *P. delalandii*, light chestnut-brown in *Phrynobatrachus*, very pale brownish-yellow in *Xenopus*. The pigmentation alone was sufficient to distinguish the tissues clearly not only immediately after the operation but even in stages with fully developed gills. In addition I stained either the donor or the host with Nile blue sulphate in some series of operations.

The transplanted tissues could be distinguished also in sections by one or more of the following characters: (1) The cells of *P. delalandii* are distinctly larger than those of the other species. (2) The granules of melanin pigment in all three ranids are finer and more numerous than in *Xenopus*. (3) The melanin pigment in *Xenopus* is present in small quantities but the pigment granules are coarser than in the ranids and found in little clusters or nests, whilst many cells are completely devoid of pigment. (4) The amount of melanin pigment in *P. adspersus* is conspicuously greater than in *P. delalandii*; this in conjunction with the smaller size of the cells in *P. adspersus* makes the tissues of these two species easily distinguishable in sections.

The present work is largely concerned with differences in size of the organs in the four species studied. It was impracticable to make the measurements in living tadpoles; the measurements were therefore undertaken either on the camera

lucida drawings (for the length of the gill filaments and the size of the oral disk) or on sections (for the adhesive organ).

DEVELOPMENT OF ADHESIVE ORGANS IN HETEROPLASTIC GRAFTS

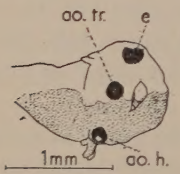
In two heteroplastic combinations the grafts were performed between species of which one has a single adhesive organ (*Xenopus*) and the other has a paired adhesive organ (*P. delalandii* and *Phrynobatrachus*). Table 1 shows the results of these operations:

TABLE 1

Development of paired or unpaired adhesive organs in heteroplastic grafts

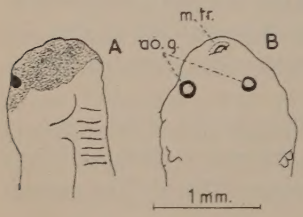
	Xenopus to *P. delalandii*	*Xenopus* to *Phrynobatrachus*	*P. delalandii* to *Xenopus*	*Phrynobatrachus* to *Xenopus*
Number of successful operations, graft lying ventrally, posterior to the mouth	11	5	5	4
One median adhesive organ developed from grafted ectoderm	7	2	0	0
Two adhesive organs developed from grafted ectoderm	0	0	1	2
Two adhesive organs developed, one from grafted ectoderm and one from host ectoderm	4	2	1	2
No adhesive organs developed from grafted ectoderm, or only atypical ones	0	1	3	0

The *Xenopus* ectoderm has in no case developed a paired adhesive organ but only a single one when transplanted to hosts normally having a pair of adhesive organs (Text-fig. 10). In the cases where two adhesive organs were produced, one from the graft and one from host ectoderm, the *Xenopus* adhesive organ tends



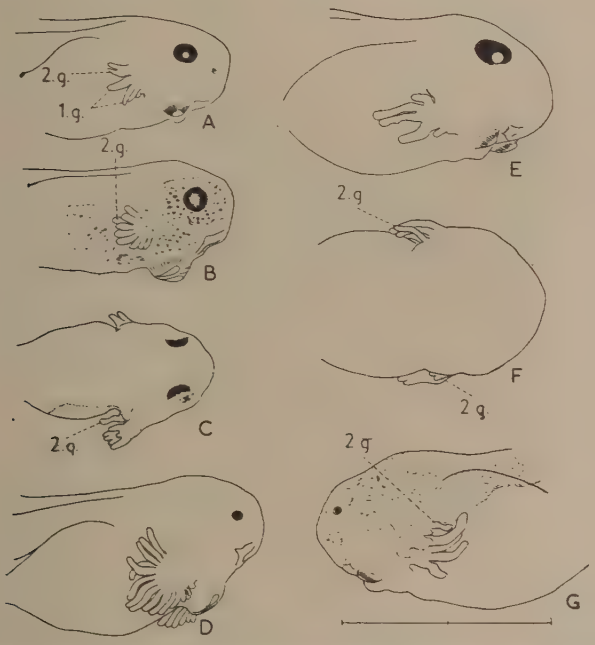
TEXT-FIG. 5. Transplantation from *Xenopus* to *Phrynobatrachus*. Epidermis of the host shown by stippling. *ao. h.*, host adhesive organ; *ao. tr.*, graft adhesive organ; *e*, eye.

to be developed in a medial position, whilst the ranid adhesive organ develops laterally (Text-fig. 5). Ectoderm of *P. delalandii* and *Phrynobatrachus* transplanted to a *Xenopus* embryo did not develop a single median adhesive organ, but a pair of adhesive organs (3 clear cases) (Text-fig. 6).



TEXT-FIG. 6. Transplantation from *Phrynobatrachus* to *Xenopus*. A: embryo 1 day after operation. Graft epidermis shown by stippling. Rudiment of graft adhesive organ visible. B: tadpole after fixation, 3 days later, in ventral view. *ao. g.*, graft adhesive organs; *m. tr.*, mouth formed by the graft.

In one heteroplastic combination the transplantation was carried out between a species having a pair of nipple-shaped adhesive organs (*P. delalandii*) and a species having a V-shaped adhesive organ (*P. adspersus*). The results are that



TEXT-FIG. 7. Reciprocal transplantations between *P. adspersus* and *P. delalandii*. A: control *P. delalandii*. B, C: *P. delalandii* host with grafted *P. adspersus* ectoderm (stippled). D: control *P. adspersus*. E, F, G: *P. adspersus* with grafted *P. delalandii* ectoderm covering mouth, adhesive organs, and right gill region completely and partially covering left gill region. (Host ectoderm stippled in G.) *1. g.*, first gill; *2. g.*, second gill.

the adhesive organs developed strictly according to the origin of the ectoderm. *P. delalandii* ectoderm developed a pair of nipple-shaped adhesive organs on a *P. adspersus* host in 3 cases (see Text-fig. 7E), and *P. adspersus* ectoderm developed a V-shaped adhesive organ on a *P. delalandii* host in 3 cases (see Text-fig. 7B). In 2 cases there is a nipple-shaped adhesive organ on one side and half a V-shaped organ on the other side (*P. adspersus* being the graft in one case and *P. delalandii* in the other case). In one case (*P. delalandii* as graft) the organ is atypical.

In all four heteroplastic combinations the adhesive organs are of different size, and I have made an attempt to find out whether the size is affected by transplanting ectoderm to a host in which the adhesive organs are larger or smaller than in the donor species. To get an estimate of the size of the adhesive organs I have made camera lucida drawings of the organs in sectioned embryos (at the same

magnification). The drawings were then measured with the aid of a planimeter, and the surface measured was used as an estimate of the size of the organ. The section used for drawing was that in which the adhesive organ appeared largest. This method of measurement is of course very approximate, and the volume of the organ or the mass of the tissue is presumably proportionate to the 3/2 power of the surface of the section. Using surfaces instead of volumes would thus introduce an error if I were to attempt to estimate changes in proportions of the organ in question. My aim, however, was only to gain information as to the presence or absence of differences in size, and I consider that the procedure adopted serves this purpose sufficiently well.

The adhesive organs are especially suitable for the study of factors determining their size, as they develop very rapidly and then remain without much change over a sufficiently long period, after which they undergo a very rapid reduction in size and degeneration. This is shown by measurements of the size of the adhesive organ in different stages of one species (Table 2). The embryos were from different batches of eggs.

TABLE 2

Size of the adhesive organ in different stages of P. delalandii
(in cm.² of camera lucida drawings of transverse sections)

Stage 16	7·5	7·7*	Stage 22	23·6	26·1
Stage 18+	17·5	19·0	Stage 23	23·9	24·6
Stage 20	19·2	19·8	Stage 24	21·4	21·4
Stage 21	22·8	23·7	Stage 25	6·6	6·7
Stage 22	22·7	—	Stage 25	5·5	8·2
Stage 22	18·6	20·3			

* Right and left side of the same embryo.

Between stages 18+ and 24 the increase or decrease of the adhesive organs does not surpass the random fluctuations as between different embryos and different batches of eggs. Most of the operated embryos were preserved in stages 22–24; those preserved when the adhesive organ had entered the retrogressive stage were not used in the following comparison.

Measurements on normal unoperated embryos show that the adhesive organ is largest in *Xenopus* ($54{\cdot}18 \pm 3{\cdot}27$). This is due partly but not entirely to the fact that the organ is unpaired and it thus corresponds to two organs in the other species. In *P. adspersus* each half of the organ was measured in the anterior section of the organ where the right and left half are separated from one another, and where incidentally the bulk of the organ is greatest. Measured in this way the adhesive organs of *P. adspersus* come next to *Xenopus* in size ($29{\cdot}73 \pm 1{\cdot}86$). The organs of *P. delalandii* are smaller than in *P. adspersus* ($21{\cdot}71 \pm 0{\cdot}85$) and those of *Phrynobatrachus* are the smallest ($10{\cdot}85 \pm 2{\cdot}30$).

It was further found that on the whole adhesive organs of operated embryos are smaller than in unoperated embryos. The operation as such, independently

of the host to which the ectoderm was transplanted, seems to affect the size of the adhesive organ. It might thus be wrong to compare the size of adhesive organs developed from grafts with those of normal embryos. As an additional control I have measured the size of organs developed from host ectoderm in cases in which the area beneath the mouth was not completely covered by the graft.

In all heteroplastic combinations the average size of the graft adhesive organs has been found to be shifted, as compared with the controls, in the direction which approximates the size of the graft adhesive organs to that of the host species. The grafts developed enlarged adhesive organs in transplantations from *P. delalandii* to *P. adspersus* and *Xenopus*, and in transplantations from *Phrynobatrachus* to *P. delalandii* and to *Xenopus*. Diminished adhesive organs developed in grafts from *P. adspersus* to *P. delalandii*, from *P. delalandii* to *Phrynobatrachus*, and from *Xenopus* to *P. delalandii* and to *Phrynobatrachus*. In single cases the size of the graft adhesive organ falls in the range of variation of the host adhesive organ. Unfortunately the range of variation is greatly increased in operated embryos, especially in respect of the graft adhesive organs, but to a lesser degree also in respect of organs developed from the host ectoderm. As a consequence the difference of the means is not statistically significant except for two heteroplastic combinations. The data for these two combinations are as follows:

P. delalandii as host, $14{\cdot}27 \pm 1{\cdot}03$ (9 cases); *P. delalandii* grafted to *P. adspersus*, $30{\cdot}22 \pm 6{\cdot}22$ (5 cases); for the difference between the means, $t = 2{\cdot}52$ for 12 degrees of freedom, $P < 0{\cdot}05$.

Xenopus as host, $35{\cdot}83 \pm 6{\cdot}90$ (3 cases); *Xenopus* grafted to *Phrynobatrachus*, $15{\cdot}25 \pm 2{\cdot}24$ (4 cases); for the difference between the means, $t = 2{\cdot}57$ for 5 degrees of freedom, $P < 0{\cdot}05$.

With these two heteroplastic combinations passing the test of statistical significance and with the corroborative evidence from other types of transplantations one can draw the conclusion that the size of adhesive organs developed from grafted ectoderm tends to approach the size normal for the host species. The size of the adhesive organ is thus not determined exclusively by the properties of the ectodermal cells of the rudiment, but also by the adjacent tissues. It is important to point out that the adjustment of the size of the graft adhesive organs was apparent at the time when they first appeared. The factors determining the size of the adhesive organs operate on the number of cells which go to form the adhesive organ and not on the rate of proliferation of these rudiments.

DEVELOPMENT OF EXTERNAL GILLS IN HETEROPLASTIC GRAFTS

In the heteroplastic combination *P. adspersus* ⇌ *P. delalandii* the shape of the early rudiments of the external gills is different in the two species, and also the number of pairs of gills: 1 pair in *P. adspersus* and 2 pairs in *P. delalandii*.

Ectoderm of *P. adspersus* transplanted to *P. delalandii* formed gills in 4 cases; in 3 of these cases the graft gills appeared as spatulate rudiments and in 1 case the gills were atypical. Subsequently the gills developed a basal lobe with a fringe of gill filaments at the edge—the typical *P. adspersus* gill (Text-fig. 7B). In the ectoderm of *P. delalandii* transplated to *P. asdpersus* the first rudiments of the gills also appeared as spatulate outgrowths in 3 cases and were atypical in 2 cases. The spatulate processes in the above 3 animals were by no means as broad as in normal *P. adspersus* embryos, but they were distinctly different from the gill rudiments of *P. delalandii*, and the gill filaments of the fully developed gills were attached to a basal lobe, as in the host (Text-fig. 7 E, G). The mode of formation of the gills found in *P. adspersus* appears thus to be determined both in the ectoderm and in the underlying tissues, and in all combinations with *P. delalandii* this character of *P. adspersus* is predominant.

The opposite is true in respect of the second pair of gills, which are normally absent in *P. adspersus*. Ectoderm of *P. adspersus* transplanted to *P. delalandii* developed a very distinct second gill in all 3 cases in which the graft gills were not rudimentary (Text-fig. 7 B, C). This proves that the ectoderm of *P. adspersus* is capable of developing a second pair of gills. The second gills were developed, however, also in 2 cases when *P. delalandii* ectoderm was transplanted to *P. adspersus* (Text-fig. 7 F, G). These second gills must have been induced by the host as they are in a correct relation to the second branchial arch of the host, and it is thus impossible to suspect that they could have been developed as a result of self-differentiation of the graft, even if the early stage of the operation had in itself not excluded the possibility of the grafted ectoderm being already determined for gill formation.

A *P. adspersus* embryo thus possesses an ectoderm competent to develop a second gill and is able to induce the formation of a second pair of gills, but does not normally do so. The only explanation of this peculiar phenomenon which occurs to me at present is that the development of the enormous first gill in a *P. adspersus* embryo in some way suppresses the formation of a second one. In the operated embryos, both in *P. adspersus* embryos with *P. delalandii* grafts, and in *P. delalandii* embryos with *P. adspersus* grafts, the gills, although essentially of the *P. adspersus* type, were in no case as large as in normal *P. adspersus* embryos. It is possible then that the dominance of the first gill was thus reduced to an extent that allowed the development of gills on the second branchial arch. I believe that it will be possible to put this explanation to an experimental test.

The number of filaments on the external gills is a further character that differs in the four species and appears to be subject to the influence of the host in heteroplastic transplantations (see Table 3).

The data show that in some of the heteroplastic combinations the number of gill filaments may approach the number found in the host. Ectoderm of *P. delalandii* has developed an excessive number of gill filaments on the first gill when transplanted to *P. adspersus*, and at the same time the number of filaments

is decreased on the second gill. Ectoderm of *P. adspersus* transplanted to *P. delalandii* developed far fewer gill filaments than in the donor species. Ectoderm of *Xenopus*, on the other hand, has not been able to develop more gill filaments on

TABLE 3

Number of filaments on the gills

The figures show the number of filaments on the first and second gill in individual cases.

Controls	*Grafts*	*Controls*
P. adspersus	*P. adsp.* to *P. del.*	*P. delalandii*
10+0	4+2	2+3
15+0	7+1	3+2
15+0	8+1	3+2
15+0	8+1	3+3
	P. del. to *P. adsp.*	3+3
	5+1	4+2
	5+1	4+3
	5+2	4+3
	9+1	4+3
Phrynobatrachus	*Phryn.* to *P. del.*	
1+1	2+1 4+2	
2+1	2+3 4+2	
2+2	3+2 4+2	
2+3	4+2 5+1	
2+3		
3+2	*P. del.* to *Phryn.*	
4+2	2+2 3+2	
4+3	2+3 3+2	
Xenopus	*Xenopus* to *P. del.*	
1+1	1+0 1+2	
2+1	1+1 1+2	
2+2	1+1 2+1	
	P. del. to *Xenopus*	
	1+1 2+1	
	2+1	
	Xenopus to *Phryn.*	*Phrynobatrachus*
	1+0 1+2	(see above)
	1+0 2+1	
	1+1	
	Phryn. to *Xenopus*	
	1+0 2+0	
	1+0	

P. delalandii and *Phrynobatrachus* hosts than it does normally. The ectoderm of *P. delalandii* and *Phrynobatrachus* transplanted to *Xenopus* developed poorly ramified gills similar to the gills of the host. The number of gill filaments in *P. delalandii* and *Phrynobatrachus* is nearly the same, so that transplantations between these two species do not present much interest.

To determine the size of the gills formed by transplanted ectoderm I measured the greatest length in millimetres of a gill filament on camera lucida drawings of

operated tadpoles (most of the drawings have been made after fixation, a few were drawings of living larvae). The results are shown in Table 4, which is arranged in the same way as the previous one. The magnification of the drawings is *circa* × 47.

TABLE 4

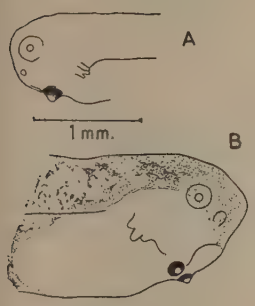
Greatest length of gill filaments

Controls	*Grafts*	*Controls*
P. adspersus	*P. adspersus* to *P. del.*	*P. delalandii*
18	9	11
18	11	11
23	12	11
26		11
	P. del. to *P. adsp.*	13
	17	18
	23	18
	23	
Phrynobatrachus	*Phryn.* to *P. del.*	
7	11 14	
8	12 15	
8	14 17	
8·5		
9·5	*P. del.* to *Phryn.*	
10	5 8	
10	7 12	
Xenopus	*Xenopus* to *P. del.*	
2·5	11	
2·5	12	
3		
3·5	*P. del.* to *Xenopus*	
4	2·5	
5·5	5	
	Xenopus to *Phryn.*	*Phrynobatrachus*
	2·5	(as above)
	4	
	5·5	
	Phryn. to *Xenopus*	
	3·5	
	6	

From the data it is hardly possible to reach any other conclusion but that the size of the gills is determined by the host. It is not only the length of the gills, however, that is so determined. The thickness of the gill filaments follows that of the host as well. This is especially obvious in heteroplastic transplantations between species which differ most in the thickness of the gill filaments, as *P. delalandii* and *Phrynobatrachus*. The result of a reciprocal transplantation between these two species is shown in Text-fig. 8. The whole pattern of the gill rudiments in cases like the ones presented in this figure is moulded after the host species.

Grafted *Xenopus* ectoderm does not conform to the pattern of the host in respect of the number of gill filaments (see above). The length of the filaments *batrachus* it developed a stomodæal invagination (8 cases out of 9 possible), but

host: two cases with relatively very long gill filaments are listed in the table. The hosts were embryos of *P. delalandii*. One of the two cases is shown in Text-fig. 9.



TEXT-FIG. 8. Reciprocal transplantation between *P. delalandii* and *Phrynobatrachus*. A: *Phrynobatrachus* host with grafted *P. delalandii* ectoderm covering practically the whole anterior part of the embryo. B: *P. delalandii* host, stained with Nile blue sulphate (shown by stippling) with grafted *Phrynobatrachus* ectoderm (white).

Xenopus ectoderm grafted to *Phrynobatrachus* hosts developed short gills as in the donor species, but possibly better results could be achieved with a greater number of experiments.

TEXT-FIG. 9. Transplantation from *Xenopus* to *P. delalandii*. A: position of graft (stippled) 1 day after operation. B, C: tadpole after fixation. Gills and adhesive organ on right side from grafted ectoderm, on left side from host ectoderm.

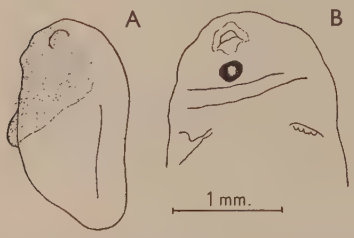
Ectoderm of *P. delalandii* and of *Phrynobatrachus* transplanted to *Xenopus* embryos developed only very short gills which were very poorly ramified. I suspect that the shortness of the gills and the incomplete development of gill filaments are correlated: a certain degree of growth of the gill rudiments may be necessary so that the formation of secondary processes, the gill filaments, may occur.

DEVELOPMENT OF THE MOUTH PARTS IN HETEROPLASTIC GRAFTS

Mouths have been developed in almost all cases in which the grafted ectoderm came to lie in the correct situation in the host embryo; the ectoderm was thus capable of reacting to the mouth-inducing influence of the host in all heteroplastic combinations.

When ectoderm of *Xenopus* was transplanted to *P. delalandii* or *Phrynobatrachus* it developed a stomodæal invagination (8 cases out of 9 possible), but

there was no trace of horny jaws, horny labial teeth, or oral disk papillae to be seen. In this respect the graft mouth retained the peculiarities of the donor species. The shape and the size of the stomodæal invagination was, however, at least in 2 cases more like that of the host (Text-fig. 10).



TEXT-FIG. 10. Transplantation from *Xenopus* to *P. delalandii*. A: position of graft (stained with Nile blue sulphate, shown by stippling) 1 day after the operation. B: after fixation, ventral view showing mouth covered by the graft and single median adhesive organ developed from graft ectoderm.

P. delalandii ectoderm developed a stomodæum in a *Xenopus* host in 3 cases out of a possible 4. Of these three mouths one has distinct horny jaws (Text-fig. 11), in another case an oral disk with labial teeth and papillae was formed, and one mouth is atypical. *Phrynobatrachus* ectoderm was in a suitable position in the *Xenopus* embryo 3 times, and developed a stomodæum in every case. The

TEXT-FIG. 11. Transplantation from *P. delalandii* to *Xenopus*. Ventral view of tadpole after fixation showing mouth with horny jaws developed from grafted ectoderm.

mouth has horny jaws in 2 cases, and in 1 case it is a simple opening, as in the host. The ectoderm of ranid embryos thus tends to produce a mouth with a typical armament of horny jaws, horny labial teeth, and oral papillae in response to an induction from a host that normally has none of these structures. Text-fig. 11 shows, however, that the size of the stomodæum is determined by the host: the horny jaws developed in the graft are only a fraction of their normal length, as can be seen by comparing Text-fig. 11 with Text-fig. 12A (drawn to the same scale).

In the heteroplastic combinations *P. adspersus* ⇌ *P. delalandii* and *P. delalandii* ⇌ *Phrynobatrachus* both partners develop horny jaws, labial teeth, and oral papillae, but the size of the oral disk differs considerably, and the factor determining this size is clearly shown by the heteroplastic grafts (see Table 5).

The data suggest that the size of the oral disk is influenced by the host. The evidence for *P. delalandii* ectoderm transplanted to *P. adspersus* is obviously insufficient, but grafts of *P. adspersus* to *P. delalandii*, and of *P. delalandii* to *Phrynobatrachus*, show a very distinct diminution of the size of the oral disk, and grafts of *Phrynobatrachus* to *P. delalandii* have developed oral disks that

are far beyond the range of variation in the donor species. The latter group of experiments is especially convincing, as a diminution of the mouth disk could have been the result of an arrest of development or of general unfavourable

TABLE 5

Breadth of the oral disk measured in mm. on camera lucida drawings of normal and operated embryos. Magnification of drawings c. × 47

Controls	*Grafts*	*Controls*
P. adspersus	*P. adsp.* to *P. del.*	*P. delalandii*
32	11	12
32	19	20
	P. del. to *P. adsp.*	
	22	
Phrynobatrachus	*Phryn.* to *P. del.*	*P. delalandii**
13	11	23
15	17	25
15·5	20	32
15·5	24	
15·5	25·5	
15·5	27	
18	31	
	31	
	P. del. to *Phryn.*	
	12	
	14	
	14	
	17	

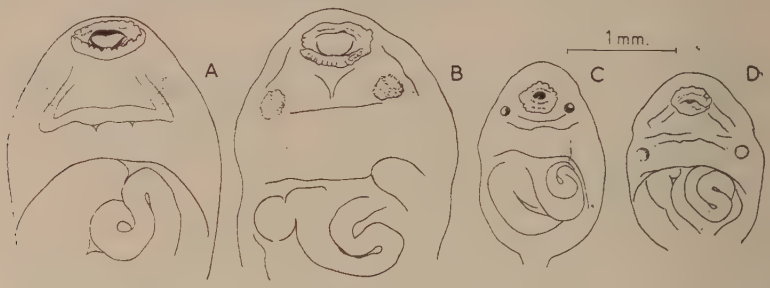
* The controls in this series are different because the operated embryos were preserved at more advanced stages of development, when the oral disk had become considerably larger. The stage of fixation of the *P. adspersus* ⇌ *P. delalandii* combination was 22–23, and for the *P. delalandii* ⇌ *Phrynobatrachus* combination 25–26, for most of the tadpoles listed in this table.

conditions, whereas an increase of size can only be due to a specific influence of the host. Text-fig. 12 shows the results of reciprocal grafts between *Phrynobatrachus* and *P. delalandii*.

The size of the mouth orifice and the length of the horny jaws covered by the grafts conform to the size of the oral disk. The thickness of the jaws, on the other hand, does not: the jaws developed from *P. delalandii* ectoderm have a much heavier lining of horn than those of *Phrynobatrachus*, both normally and in the graft (Text-fig. 12). The mouth developed from *P. delalandii* ectoderm on a *Phrynobatrachus* tadpole appears as if the coarser histological elements of the donor species have been squeezed into a space that is too small for them. The reverse can be found in respect of jaws formed by *Phrynobatrachus* ectoderm.

The number and arrangement of rows of horny labial teeth on the mouth disk of the tadpole is one of the best characters for distinguishing the tadpoles of different species. The three species of ranids used for my work all differ in this

respect. It was hoped therefore that the heteroplastic grafts would show whether these systematic characters are inherent in the ectoderm or are determined by induction from the underlying parts. Unfortunately I have not been able to rear



TEXT-FIG. 12. Reciprocal transplantation between *P. delalandii* and *Phrynobatrachus*. A: *P. delalandii* control. B: P. *delalandii* host with mouth and adhesive organs developed from grafted *Phrynobatrachus* ectoderm. C: *Phrynobatrachus* host with mouth and adhesive organs developed from grafted *P. delalandii* ectoderm. D: *Phrynobatrachus* control.

the tadpoles to a sufficiently late stage, and although labial teeth differentiated in *P. delalandii* grafts, the full number of rows necessary for identifying the species was not developed.

A different feature of the labial teeth has, however, come to my notice. At stage 25 when the tadpole is fully formed, and the external gills are concealed by opercular folds, the tadpoles of *P. delalandii* already possess labial teeth, whereas *Phrynobatrachus* tadpoles do not. I also found this to be true in the reciprocal grafts: *P. delalandii* ectoderm produced teeth by stage 25, and *Phrynobatrachus* ectoderm did not do so (see Text-fig. 12). On sections of *Phrynobatrachus* tadpoles in stage 25 one can see the rows of enlarged cells which produce the labial teeth, and similar rows of cells can be found in the mouth disks developed from *Phrynobatrachus* grafts. Each species has thus kept its own rhythm of differentiation.

DISCUSSION

The results of my experiments may be considered from two viewpoints. On the one hand, they supply information as to the mode of development of each of the three organs studied. On the other hand, they throw some light on the main problem set out in the introduction: on the factors determining the development of distinctions between different species of animals.

The adhesive organs. According to Holtfreter (1935*b*, 1938) the adhesive organs differ in their mode of origin from the other rudiments developing from the ectoderm in amphibian embryos. The development of the latter is induced by parts of the endo-mesoderm towards the end of gastrulation and in the following stages. The adhesive organs, on the other hand, are supposed to develop by

self-differentiation in the absence of specific influences of the adjoining parts. That the development of adhesive organs in normal embryos is restricted to the position behind the mouth is due, according to Holtfreter, to the rest of the ectoderm differentiating along other lines under the influence of local inducing stimuli. Holtfreter's view was supported later by Detlaff (1945) and Bordzilowskaja & Shuliak (1948), who found that a contact with mesoderm prevents the formation of adhesive organs, and they develop only in the region (ventral to the mouth) where there is no mesoderm, and the ectoderm is in contact with endoderm.

On the other hand, Schotté & Edds (1940) and Chen & Baltzer (1951) found that in heteroplastic grafts of anuran ectoderm to urodele hosts the adhesive organs develop only in specific positions, ventral to the mouth or lateral to the mouth at the site of the balancer. They conclude that the development of the adhesive organ is a result of positive inducing stimuli existing in these two positions. In the balancer region the induced adhesive organs are underlain by mesoderm, which contradicts the results of Bordzilowskaja & Shuliak. As to Holtfreter's conclusions, they are based on the development of fragmentary parts of embryos which are considerably disorganized, and in these conditions not only the adhesive organs but also many other structures may develop by a self-differentiation which is contrary to the normal prospective significance of embryonic parts (compare Bautzmann, 1929).

In my experiments the adhesive organs in grafts developed in the position in which they would normally develop in the species to which the grafted ectoderm belonged. This suggests that their localization was determined by the position of the grafted cells in the host. In one case only did I find a graft adhesive organ at an abnormal distance from the mouth on the ventral side of the embryo. There was, however, a piece of brain tissue developed from the graft in the immediate vicinity of the adhesive organ, and the brain tissue may have induced this heterotopic adhesive organ; a similar induction has been described by Schmidt (1937*d*).

Although the graft adhesive organs developed in the correct position according to the donor species, the position was not always correct in respect of the host species. This was especially the case in transplantations between *Xenopus*, which has an unpaired median adhesive organ, and ranid species having a pair of adhesive organs situated laterally. In no case (except for the one case of heterotopic development mentioned above) has a *Xenopus* adhesive organ developed from the graft in any other location than just behind the mouth, ventrally, that is in its normal position. It follows that the place of development of the adhesive organ has been determined by local factors. The local factors in a ranid embryo, however, induce host ectoderm to differentiate adhesive organs in a different position: near the angle of the mouth. The grafted *Xenopus* ectoderm is thus capable of converting the inducing influence of the host in such a way as to cause it to serve as an inductor of an adhesive organ which is heterotopic in respect of the host as a whole.

The same argument applies to the development of a pair of adhesive organs from *P. delalandii* or *Phrynobatrachus* ectoderm grafted to a *Xenopus* embryo: whilst the differentiation of an adhesive organ is clearly due to a local influence, as it is strictly in correspondence to the normal location of the organ in the donor species, this local influence is transformed by the reacting material so as to become the cause of the development of the organ in positions that are abnormal in respect of the host.

The question may arise whether the median adhesive organ and a pair of lateral ones develop in response to the same inducing stimulus or to different ones. The latter would appear to be highly improbable. As an alternative explanation I can only suggest that the primary inductive stimulus causes the formation of a rudiment stretching across the midline behind the mouth (as in the V-shaped rudiment mentioned before), and that later the cells of this rudiment, depending on their specific affinities (Holtfreter, 1939; Weiss, 1947), either concentrate towards the midline (in *Xenopus*) or draw apart towards the angles of the mouth (in *P. delalandii* and *Phrynobatrachus*).

The same explanation applies to the results of heteroplastic transplantations between *P. adspersus* and *P. delalandii* in which ectoderm of each species developed adhesive organs in a position that corresponds strictly to the peculiarities of the donor species. As the adhesive organ of *P. adspersus* is situated more medially than in *P. delalandii* and is actually continuous across the median plane, it may be said to retain the original position in which the adhesive organ rudiment is induced, whilst adhesive organs induced in grafted *P. delalandii* ectoderm separate into two lateral structures.

The external gills. The development of the external gills in the *Urodela* has been very thoroughly investigated and it has been shown that they are in the last instance induced by the endodermal gill pouches (see Harrison, 1921; Severinghaus, 1930; Ichikawa, 1934). When the endoderm of the gill region is removed, the external gills do not develop (Balinsky, 1948). The mechanism of the development of the gills in the *Anura* is not known so well. Ekman (1922) transplanted presumptive gill ectoderm in anuran embryos and found that gills could develop heterotopically by self-differentiation. As the transplantations were performed on embryos after the end of gastrulation the gills might have been determined earlier under the influence of the endoderm. Schmidt transplanted presumptive ventral ectoderm of a *Bombinator* gastrula to the side of a very early neurula of *Triturus* and found that grafted ectoderm developed external gills in the gill region of the host. This shows that the gills may be induced by local tissues. The position of the induced gills, however, did not correspond exactly with the position of the host's gill clefts, thus showing a certain degree of 'autonomy' (Schmidt, 1936, 1937*b*). The gills are described as 'intermediate between the donor and the host'. They were rather atypical and soon degenerated.

Although in my experiments the graft gills were abnormal in many cases, they

were quite well developed in some embryos. In all the heteroplastic combinations I have observed cases of very far-reaching development of the gills, showing the compatibility of ectoderm and mesoderm, the latter of which produced the core of the gill. The presence of blood-vessels and blood circulation in these gills should especially be noted. In every case the gills developed in the correct position in respect of the branchial region of the host. There can hardly be any doubt therefore that the gills are induced by adjoining parts in the host. My experiments do not give any indication whether the endoderm is the inducing part.

It has been shown previously that an organism may possess the ability to induce an organ which it does not normally develop itself: an *Amblystoma mexicanum* embryo normally has no balancer but can induce one if *Triturus* ectoderm is grafted in the position in which a balancer develops (Mangold, 1931; Rotmann, 1935). The presence or absence of an organ is here determined by the properties of the reacting ectoderm. A somewhat similar case is presented by the *P. adspersus* embryo which does not develop a second pair of gills, but induces a second gill in *P. delalandii* ectoderm grafted to the gill region of a *P. adspersus* embryo. The position is, however, complicated by the further fact that *P. adspersus* ectoderm develops a second gill when transplanted to a *P. delalandii* embryo. The ectoderm of *P. adspersus* is thus capable of second gill development. Although in *P. adspersus* there exists the necessary inducing stimulus, the second gill is not formed in normal development. There must be some factor in a normal *P. adspersus* embryo which does not allow the second pair of gills to develop. I have suggested above that the excessive development of the first gill might possibly account for an inhibition of the second one. Whether this is the correct explanation or not, the absence of the second gill in *P. adspersus* seems to be the result of properties of the whole system, and not to be caused by the reactive abilities of the ectoderm.

The mouth parts. It is now generally recognized that the ectodermal mouth invagination is induced by the endoderm of the mouth region when it comes in contact with the presumptive epidermis at the end of gastrulation (Ströer, 1933; Holtfreter, 1935*a*, 1935*b*; Balinsky, 1939). The results of the present experiments are in full agreement with this view.

I can now discuss the main problem towards the solution of which the present experiments were expected to contribute, namely, whether differences in the inducing or the reacting systems are mainly responsible for the distinctions between related species of animals. The experiments which I have described in the preceding pages suggest that the phenomenon is rather complicated as both agents (the inductor and the reacting material) may be responsible for the determination of different properties of even one and the same organ. It is therefore necessary to undertake a more detailed analysis of the morphogenetic processes involved.

I believe that a satisfactory survey of all the facts may be given if we distinguish between what I would like to call histogenetic and organogenetic processes.

The histogenetic processes are those which are concerned with the elaboration of types of cells with specific morphological and physiological properties, and with the mutual arrangement of the cells. Following Holtfreter (1939) and Weiss (1947), I presume that the arrangement of the cells in the tissues is an expression of their affinities, and thus a result of their physiological characteristics. The affinities of the cells must be responsible not only for the arrangement of cells in simple tissues (simple or stratified epithelium, mesenchyme, &c.) but also for their positions in simple, discreet structures consisting of a small number of cells, such as taste buds, renal tubules, or acini of glands.

Organogenetic processes are those which are concerned with the mobilization of the necessary numbers or masses of specifically differentiated cells (or cells capable of a specific differentiation) in certain positions with respect to the animal as a whole or with respect to other parts of the animal's body.

The histogenetic processes would presumably be based directly on the genotype of the animal. If a specific type of cell is not in the repertoire provided for by the genotype it cannot be developed. Hence in heteroplastic transplantations the histological structures should be expected to be always determined by the donor species of the cells concerned.

A very clear-cut example of histogenetic processes in my experiments is the differentiation of labial teeth and horny jaws. The elaboration of horn can only occur if the cells of the animal possess the enzymic mechanism that is instrumental in the chemical transformations involved. The enzymic mechanisms of the cells are probably directly dependent on the genotype; it is therefore impossible for cells of *Xenopus* to develop horny teeth or horny jaws, because its genotype does not allow for the necessary chemical transformations. (The possibility that *Xenopus* may produce keratin after metamorphosis does not invalidate the above statement, because the mechanisms may not be available in the early larval stage.) The induction of horny teeth and horny jaws in ranid ectoderm transplanted to a *Xenopus* host, on the other hand, shows that the inductive stimuli emanating from the deeper lying parts in the mouth region are the same or similar in all frogs and therefore evoke the same reaction as would occur in normal development (in ectoderm that is not transplanted to a different host).

The shape of the adhesive organ appears to be another character which is due to the properties of cells and to their affinities. In *P. delalandii*, *Phrynobatrachus*, and *Xenopus* the cells of the adhesive organ rudiment arrange themselves into a conical or nipple-shaped mass with a roughly circular circumference. In *P. adspersus* the cells of the rudiment are arranged in a V-shaped mass. As this is approximately the disposition of the rudiment in the earliest stage of its development, it may be permissible to suggest that the cells in *P. adspersus* are less capable of movement, and are thus 'frozen' in their original positions. The

differences in the shape of the adhesive organs would thus be the result of properties of the individual cells.

The concentration of the secretory cells of the adhesive organ in one median mass (in *Xenopus*) or in two lateral masses (in *P. delalandii* and *Phrynobatrachus*) may be due to the different affinities of the cells of the rudiment, as indicated on p. 112. The influences of the surroundings in both cases are identical, as *Xenopus* ectoderm forms a single adhesive organ on a ranid host, and ranid ectoderm develops a pair of adhesive organs when transplanted to a *Xenopus* embryo.

If the processes of cellular differentiation are under the control of the genes and the enzymic systems elaborated through the action of the genes, then it would be expected that the rhythm of such processes would be largely inherent in the cells. A striking corroboration of this postulate is given by the development of the labial teeth in *Phrynobatrachus* ectoderm. In this species the development of the labial teeth is retarded as compared with other ranid tadpoles, and this retarded development has been preserved after transplantation to a species with a normal rhythm (*P. delalandii*). The induction causing the development of the labial teeth must have occurred at the time when the labial teeth of the host are induced, but the reaction in the form of differentiation of horny teeth could only proceed in the rhythm determined by the genotype of the reacting cells.

The organogenetic processes, at least in veterbrate animals, depend largely on the correlations between parts of the embryo. The dependence on the genotype is an indirect one, even though it may be correct that these processes are also determined by the genes in the last instance. Given the possibility of developing a certain type of cell, the number of cells of one type and their position in the embryo need not be determined by the genotype, but by epigenetic factors, of which not all are strictly genotypical.

We may presume, for instance, that the ectodermal cells forming the epithelium of the external gills belong to one specific type. The development of hollow protrusions (the external gills) is somehow dependent on the physiological and morphogenetical properties of this cell type. The acquisition by cells of the properties of external gill epithelium is caused, as I have mentioned earlier, by an induction from the endodermal gill pouches. Supposing that the ability of a cell to become a component part of an external gill depends on the possession of a certain genotype, and taking into consideration that the genotype is probably the same in all the cells of an individual, we would expect that external gills would develop at any site in which the environmental conditions is of a suitable nature. In other words, a genotype which provides for the formation of one gill or one pair of gills (as in *P. adspersus*) provides at the same time for the development of another pair of gills, if the environmental conditions should favour such a development. Thus when a second pair of gills is induced in *P. adspersus* ectoderm transplanted to a *P. delalandii* host, no new type of cell is created, but the same type of cell (external gill epithelium cell) is caused to differentiate in two pairs of areas instead of in one pair of areas. There is thus

an essential difference between the induction of a second gill in a species which normally possesses one pair of gills and the induction of a balancer in a species which does not possess one: no new type of cell is developed in the first case, whereas the cells of a balancer may be presumed to be a special type of cell, and it is this special type of cell which cannot be produced in certain genotypes (or may be produced only under exceptional conditions).

Again the size of a gill rudiment depends on the number of cells (or the mass of cells) which undergoes differentiation of a certain type. An increase of the rudiment does not necessitate the elaboration of a different type of cell, and can be achieved by transplanting competent material to a different host in which the induction causing the differentiation of one particular rudiment is spread over a greater area than in the donor species. The same holds good, *mutatis mutandis*, for a diminution of the size of the rudiment in heteroplastic grafting. Both cases are realized in the development of gills and adhesive organs in heteroplastically transplanted ectoderm, contrary to Rotmann's statement (1939) that transplanted ectoderm can give rise to organs which are smaller than normal for the species, but not to organs that are larger than the normal ones. Rotmann's statement is based on his investigations of lens development, and it may possibly be true of that organ.

Perhaps the clearest case of decrease and increase of the size of rudiments caused by transplantation of ectoderm to a different host is to be found in the development of the mouth as shown by my experiments. Mouths developed from *Phrynobatrachus* ectoderm grafted on to the considerably larger *P. delalandii* host have exceeded by far the size of the mouth in the donor species, and have attained normal proportions with respect to the host. This example is of special interest because it is fairly certain that the ectodermal mouth invagination is induced by the oral endoderm when it comes into contact with the ectoderm. The number of cells acquiring various differentiations found in the mouth parts would thus depend directly on the surface of contact between endoderm and ectoderm (other factors being equal). No new types of cells being required, there is no hindrance to an increase or decrease of the organ as a whole. It has been noted, however (p. 109), that the components of the mouth in heteroplastic grafts are not diminished or increased proportionally to the organ as a whole: the horny jaws are too thick for a diminished mouth, the labial teeth and the oral papillae preserve the size normal for the donor species. This shows that the size of these component parts is directly determined by the properties of the cells, and hence by the genotype.

The length of the gill filaments in gills developed from heteroplastic grafts should not be confused with the size of the gill rudiments. The elongation of the gills and formation of the gill filaments may involve three types of morphogenetic process: (1) evagination of part of the ectodermal epithelial layer, (2) stretching of the evaginated part, and (3) growth of the evaginated part. The initial size of the evaginated part would probably depend on the area participating in the

evagination, and thus on the number of cells acquiring a specific type of differentiation. The other two factors are not necessarily dependent on the first one. Growth in particular is a complex morphogenetic factor which has to be taken into account separately. Harrison (1929) has proved that the growth of heteroplastically transplanted parts may be influenced by the host. Although the growth rests on intracellular reactions, and thus should be determined by the genotype and fall amongst the histogenetic processes, it is known to be subject to environmental conditions (heteromorphic growth of Schmalhausen, 1927).

That the length of the gill filaments in my heteroplastic grafts was determined mainly by the host makes it probable that apart from the number of cells mobilized in each case for gill formation, the other components of gill formation, stretching and growth, are also controlled by underlying tissues and not by the ectodermal epithelium. As the gills do not develop and elongate normally if they fail to be supplied by blood-vessels, it would seem very probable that the elongation of the gills is determined by the mesodermal tissues which enter into the formation of the gills.

Applying the distinction between histogenetic and organogenetic processes to the facts found in the literature I find that it helps to solve some contradictions and to bring them under one common viewpoint.

The development of a fin fold on the trunk region or even on the head does not involve the elaboration of a different type of cell from that participating in the formation of a fin fold on the tail. The development of the fin fold is thus a clear-cut organogenetic process, and the extent of the fin fold is dependent only on the area covered by the inducing influence. What changes from species to species is the extent of this area. This obviously involves a gradient of some kind, but how the gradient links up with the genotype falls outside the scope of this paper.

Rotmann has found (1933) that if the limb mesoderm is covered by ectoderm of a different species (in reciprocal grafts between *Triturus cristatus* and *Triturus taeniatus*) the shape of the limb and the length of the fingers is determined by the host. It does not require a different type of epithelial cell to cover a long finger from what it does to cover a short finger. Therefore the genotype of the ectoderm does not have any influence on finger length.

The same author (Rotmann, 1934) discovered that if heteroplastic grafts in *Triturus taeniatus* ⇌ *Triturus cristatus* transplantations lined the stomodæal invagination, the arrangement of the teeth is hostwise, although the enamel organ of the tooth is derived from graft ectoderm. The same observation was made by Woerdeman (Woerdeman & Raven, 1946) in transplantations between *Triturus taeniatus* and the Axolotl, with the interesting addition that the size of the individual teeth was according to the origin of the ectoderm. The size of the teeth is thus determined by the properties of the epithelial cells; this is a histogenetic process, resting directly on the genotype of the cells. The mobilization of the ectodermal cells for differentiation as cells of the enamel organ is performed

through correlations with other parts (the skeletal parts of the skull) and is thus an organogenetic process.

The answer to the question put forward at the beginning of this paper may thus be given as follows:

The differences between related species are always dependent on the reacting material in characters that are concerned with the fine structure of cells and their arrangement (histogenetic processes). In characters that are concerned with the extension and position of areas differentiated in certain ways (organogenetic processes) the differences between related species may be due to a modification of the inducing systems.

SUMMARY

1. The tadpoles of four species of *Anura* used for the experiments (*Pyxicephalus adspersus*, *Pyxicephalus delalandii*, *Phrynobatrachus natalensis*, and *Xenopus laevis*) differ in the structure of their adhesive organs, external gills, and mouth parts, as well as in size and other properties.

2. To investigate whether the distinctions in the above organs are due to different reactive abilities of the ectoderm or to differences in inducing systems, parts of the presumptive ectoderm were transplanted in the early gastrula stage from one species to another.

3. Adhesive organs, gills, and mouth parts were developed from grafted ectoderm in conformity with its position in the host embryo, as a result of inducing influences from the underlying parts of the host. The organs developed from the grafts partly retained the characters of the donor species and partly acquired the characters of the host species.

4. In respect of all characters concerned with the differentiation of special types of cells and with their mutual arrangement the graft ectoderm strictly retained the peculiarities of the donor species. These characters were: presence or absence of horny jaws and horny labial teeth; rhythm of differentiation of the labial teeth; shape of the adhesive organ; subdivision of the rudiment of the adhesive organ into two lateral organs or retention of an organ that is continuous across the midline (i.e. is single and median); branching of the external gills. It is proposed to distinguish processes of this type as histogenetic processes. They are presumably based directly on the genotype of the cells.

5. The size of all induced graft structures, such as the mouth, the gill rudiments, or the adhesive organ, showed various degrees of approximation to the size of corresponding organs of the host, and this not through retarded or accelerated growth but through an increased or decreased initial size of the rudiments. Ectoderm of a species having only one pair of gills developed gills of a second pair when transplanted to an embryo having normally two pairs of gills. The length of the gill filaments was predominantly determined by the host.

6. Processes controlling characters of this second type, that is characters

involving the number or mass of cells and the position of areas differentiating in a specific way, I call organogenetic processes. Differences in organogenetic processes between related species may thus be due to a modification of the inducing systems and not to changes in reactive abilities of cells.

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The Effects of Ultrasonic Treatment on Embryonic Development of *Drosophila melanogaster*

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WITH TWO PLATES

INTRODUCTION

THE work of Seidel and his school (reviewed Seidel, 1935, 1936) showed that embryonic development within the class Insecta ranges from the indeterminate or regulative type in the Odonata to completely determinate or mosaic development in the Diptera. Between these two extremes are insects in which the developmental fate of the egg is not determined at the time of fertilization, but which becomes determined at varying times afterwards. Actually the scale is a relative one, for development eventually becomes completely determined in the Odonata, and there is experimental evidence (Howland & Sonnenblick, 1936) which suggests there is some regulative power in the Dipteran egg prior to fusion of the gamete nuclei.

Experimental methods used to such great advantage in studies of vertebrate development cannot be used in studying the insect egg, as the turgor pressure and fluidity of the egg contents make transplantation studies and the culturing of embryonic tissues *in vitro* impossible in most forms. The many alternative techniques which have been devised for studying embryological processes in insects have been reviewed by Richards & Millar (1937) and Krause (1939).

Many workers have studied the effects of treatment applied to the egg in later post-embryonic developmental stages; for example Howland & Child (1935) and Howland & Sonnenblick (1936) studied the effects of embryonic punctures on development of structures in the adult, and Geigy (1931*a*) the effects of ultraviolet irradiation of the egg on imaginal structures. Waddington (1942) has pointed out the dangers of using defect experiments as a means of discovering developmental potentials.

Experimental methods which have been used in purely embryological studies on the Diptera include:

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1. Cauterization (Reith, 1925—*Musca*; Strasburger, 1934—*Calliphora*; Howland & Robertson, 1934—*Drosophila*).
2. Irradiation with ultraviolet light (Geigy, 1931*b*—*Drosophila*; Aboim, 1945—*Drosophila*).
3. Constriction (Pauli, 1927—*Calliphora* and *Musca*; Rostand, 1927—*Calliphora*).
4. Centrifugation (Pauli, 1927—*Calliphora* and *Musca*; Howland, 1941—*Drosophila*).
5. Treatment with X-rays (Sonnenblick, 1940—*Drosophila*; Sonnenblick & Henshaw, 1941—*Drosophila*).

Although these methods have given us much valuable information about embryonic development in Diptera (especially the experiments of Reith and Pauli which demonstrated the mosaic nature of development, the totipotency of cleavage nuclei, and the importance of the periplasm in determining the fate of nuclei which enter various regions of it) they are not completely satisfactory tools. Microcautery and radiation treatments result in the destruction of regions of the egg; the chromosomes are also affected by the use of ionizing radiations. Constriction experiments are tedious and difficult because of the size of the egg, and are infrequently successful. Centrifugation experiments require treatment of the developing eggs for long periods of time even at high speeds to shift the periplasm.

The use of ultrasonic waves as an experimental method in insect embryology has not yet been fully exploited. Ultrasonic treatment may cause a 'stirring round' in certain regions of the egg but no nuclei need be destroyed. Fritz-Niggli (1950, 1951) has studied the effects of ultrasonics on development in *Drosophila*, but although all developmental stages were treated, her histological studies were confined to prepupal, pupal, and adult stages. A review of biological studies with ultrasonics is given by Dognon, Biancani, E., & Biancani, H. (1937), and an account of the effects of ultrasonics on mitosis by Selman (1952). The apparatus used in these experiments was similar to that developed by Selman & Wilkins (1949) for the treatment of biological material under controlled conditions. It was found that when the early developmental stages of *Drosophila* embryos were treated for short periods with ultrasonic waves at intensities low enough to obviate the danger of cavitation and chemical damage, the subsequent embryonic development was abnormal. A preliminary report of these experiments has already been published (Selman & Counce, 1953).

TECHNIQUE

Eggs were collected at half-hour intervals from rapidly laying *Oregon K* flies of stock maintained in this laboratory, and were transferred to the central region of thin agar disks for storage in Petri dishes under moist conditions at 25° C. Immediately before treatment the agar disks were placed in the treatment vessel so that the eggs faced the ultrasonic generator (Text-fig. 1). The ultrasonic waves

at a frequency of 1 Mc./sec. were applied through a water medium maintained at room temperature, using a uniform beam of ultrasonics of known intensity. The ultrasonic intensities were known from a calibration chart for the generator, previously prepared from measurements made similarly to those described by Selman & Wilkins (1949). After treatment the eggs were replaced in the Petri dishes for further incubation at 25° C. until they were needed for fixation.

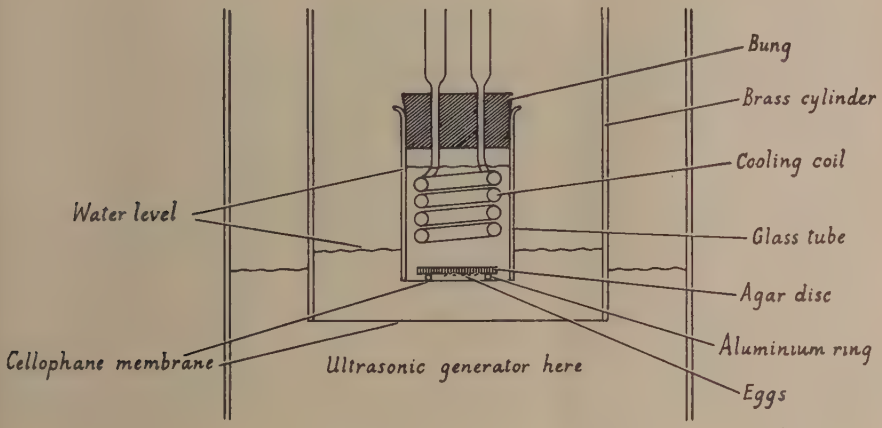


DIAGRAM OF ULTRASONIC TREATMENT VESSEL

TEXT-FIG. 1.

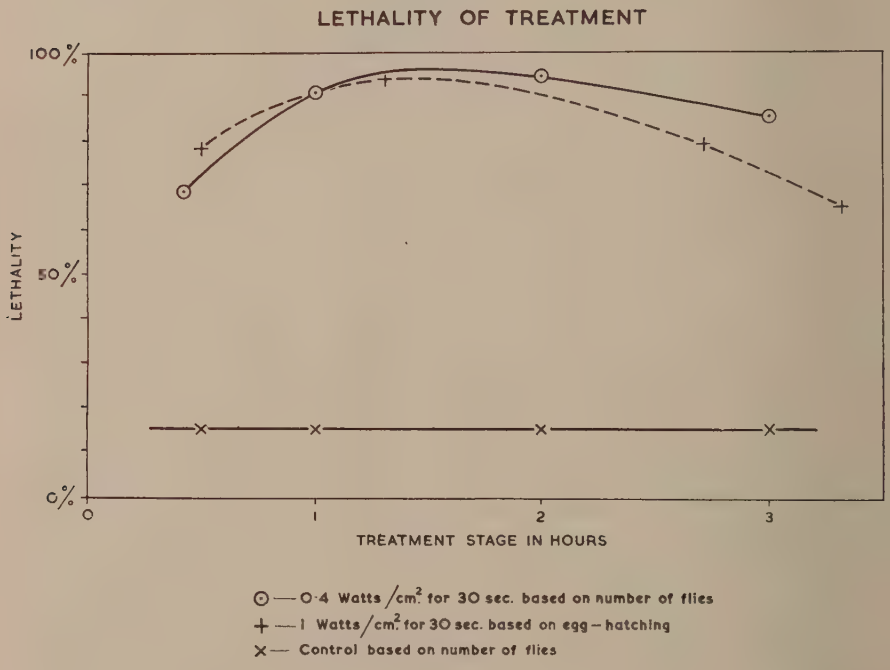
A 6 : 16 : 1 formol : alcohol : acetic acid mixture was used as fixative (Darlington & La Cour, 1942, after Smith). To ensure rapid and even fixation the vitelline membrane was pierced with the tip of an exceedingly fine tungsten needle as soon as the egg was placed in the fixative. After fixation for 2 to 24 hours, eggs were dehydrated through a butyl alcohol series (Darlington & La Cour, 1942) containing eosin, infiltrated with 45° C. paraffin with an equal amount of phenol, changed after 12 to 16 hours into fresh 45° C. paraffin and embedded in hard wax. Serial sections were cut at 4 microns and stained in Heidenhain's iron haematoxylin according to the schedule suggested by Sonnenblick (1950).

None of the eggs treated in the main experiment were dechorionated. The optically opaque chorion was transparent to ultrasonics and did not hinder fixation, while the chorionic filaments were useful guides to the orientation of abnormal embryos. In subsidiary experiments, however, dechorionated eggs were treated with ultrasonics using a special small generator mounted on a microscope stage (cf. Harvey, E. N., Harvey, E. B., & Loomis, 1928) and this allowed direct visual microscopic examination of the living egg to be made by

transmitted light while the ultrasonic treatment was actually being given. In this way disturbances caused by the treatment could be seen. With this apparatus, however, it was not possible to determine precisely the intensity of the ultrasonic waves which were applied, nor was there any temperature control, and for these reasons the microscope stage generator was not used in the main embryological or cytological studies.

METHOD AND RESULTS

Preliminary experiments showed that treatment at any particular ultrasonic intensity for a given time produced a maximum lethality when the eggs were at the stage of migration of the nuclei to the periphery and the formation of the



TEXT-FIG. 2.

syncytial blastoderm (Text-fig. 2). This was the case when the lethality of the treatment was reckoned by counting the numbers of eggs which hatched or failed to hatch, and also from estimates based on the subsequent numbers of flies.

In the main experiment the following early developmental stages were treated with ultrasonics: maturation or early cleavage (immediately after collection); late cleavage stage ($1 \pm \frac{1}{4}$ hr. after deposition). Several ultrasonic intensities were used: 0·05, 0·1, 0·3, 0·5, and 1·2 watts/cm.^{2}; all treatments were of 30 seconds

duration at room temperature. Many eggs were treated simultaneously so that eggs from the same batch could be fixed at a number of times after treatment.

Table 1

Number and classification of eggs fixed after treatment at different ultrasonic intensities

Intensities of ultrasonic treatment are given in watts/cm.^{2} ± 0·1. Age at treatment quoted in hours. *N*, normal; *A*, abnormal; *ND*, no development; *VA*, very abnormal; *SA*, slight abnormalities; *AO*, abnormal organization; *D*, differentiation without organogenesis; *P*, proliferation without differentiation; *U*, unfertilized.

Intensity	Age at treatment	Ages at time of fixation																	
		0–1 hr.		1–2 hr.		2–3½ hr.			3½–10 hr.				12–20 hr.						
		N	*A*	*N*	*A*	*N*	*A*	*ND*	*N*	*A*	*VA*	*ND*	*N*	*SA*	*AO*	*D*	*P*	*ND*	*U*	*Total*
0·05	2	—	—	—	—	5	0	0	—	—	—	—	—	—	—	—	—	—	5	10
0·1	½	—	—	—	—	3	3	—	—	—	—	—	2	1	0	0	0	2	0	11
0·1	1	—	—	1	1	—	—	—	1	2	0	1	—	—	—	—	—	—	0	6
0·1	2	—	—	—	—	5	5	—	3	3	0	0	3	3	0	0	0	0	2	24
0·3	½	3	8	—	—	4	2	—	2	4	0	2	13	10	0	0	0	6	0	54
0·3	1	—	—	3	6	1	1	4	—	—	—	—	2	1	0	0	0	10	0	28
0·3	2	—	—	—	—	4	8	0	6	17	28	6	12	7	18	10	5	4	2	127
0·5	½	3	3	—	—	5	2	—	—	—	—	—	4	4	0	0	0	0	0	21
0·5	1	—	—	1	6	—	—	—	0	1	1	0	1	1	0	2	2	0	0	15
0·5	2	—	—	—	—	5	8	—	6	9	9	0	11	3	5	8	5	3	1	73
1·2	½	0	4	—	—	0	0	2	—	—	—	—	3	0	1?	0	0	8	0	18
1·2	1	—	—	0	4	—	—	—	—	—	1	5	—	—	—	—	1	2	0	13
1·2	2	—	—	—	—	1	11	—	1	4	6	1	0	0	4	2	1	0	2	33
Totals		6	15	5	17	33	40	6	19	40	45	15	51	30	28	22	14	35	12	433

Table 1 shows the numbers of eggs treated at each developmental stage and at each ultrasonic intensity, together with a classification of the subsequent conditions of development reached by the embryos at the time of fixation. Table 1 was prepared from microscopic examination of permanent stained serial sections.

Ultrasonic intensities lower than 0·1 watt/cm.^{2} had little or no effect on embryonic development irrespective of the stage at the time of treatment.

Treatment of preblastoderm stages

When ultrasonic waves of intensity about 0·5 watts/cm.^{2} were applied during cleavage stage to eggs mounted on the microscope stage generator, a slow rotary movement of a large proportion of the central region of the egg was observed; the yolk granules scattered sufficient light for these movements to be followed. The periplasm was more resistant to displacement, for its position remained unaltered. If the treatment were stopped temporarily and then restarted again, a higher intensity was required to set the central region in motion once more.

Eggs treated during preblastodermal stages at intensities 0·3, 0·5, and 1·2 watts/cm.^{2} and fixed immediately showed clearly the outline of the disturbed regions (Plate 1, fig. 1). The staining reactions of the two regions of cytoplasm differed, those parts which had been moved staining more lightly than adjacent

regions; the disturbed parts were also without yolk granules at their periphery. The cytoplasm of the egg was more vacuolar than normal.

Treatment prior to blastoderm formation usually had one of two results: either the ooplasmic contents were so disordered that reconstitution did not take place and the egg subsequently degenerated without further development, or reconstitution occurred and development was normal but slightly delayed (see Table 1). All eggs treated at this stage at an intensity of 1·2 watts/cm.^{2} failed to show any sign of organization subsequently. They exhibited abnormal cytology (see later section) which may have caused death, but degeneration set in before any sign of blastoderm formation could be seen. The one embryo treated at this intensity in which abnormal organogenesis was observed (Table 1) was in all probability at a somewhat later stage in development than the other eggs treated at this time. Eggs collected from rapidly laying females are usually at the same stage of development at the time of deposition (pre-fusion of the gamete nuclei), but occasionally eggs may be retained in the uterus of the female for varying periods of time after fertilization. The development of such eggs continues in the uterus, and they may even be deposited just before the emergence of the larva.

After treatment at lower intensities the slight abnormalities observed in embryos in which organogenesis had been completed were usually related to abnormal distribution of yolk granules. These were found in the proventriculus, although they do not normally occur there; in other instances abnormally large quantities of yolk were included in the nervous system. Some abnormalities in external segmentation were also observed. It is not certain whether those embryos showing slight abnormalities might not have developed into adult flies. It is possible that embryos of this sort account in part for the delayed mortality observed by Fritz-Niggli (1950, 1951), which was confirmed by us during the course of this work. Such embryos may emerge from the embryonic membranes only to die at a later stage in development, but there is no proof of this.

Treatment of syncytial blastoderm stages

Using the ultrasonic microscope-stage generator it was observed that the pole cells were the part most easily set in motion when embryos were treated at the syncytial blastoderm stage. Intensities of 0·3 to 0·5 watts/cm.^{2} caused them to spin rapidly and the disturbance frequently caused a break in the posterior region of the blastoderm through which yolk and cytoplasm from within the interior of the embryo could be seen to flow. There was also a slow progression of blastoderm nuclei and cytoplasm towards the area of disturbance. In a few cases small air-bubbles in the water on the outer surface of the embryo were caused to vibrate in the ultrasonic beam and gave rise to secondary disturbances. Those breaks which occurred in the syncytial blastoderm, not at the posterior end, were probably produced in this way.

Treatment at the syncytial blastoderm stage was found to give rise to the greatest proportion of embryos showing abnormal development. At this stage

ultrasonic intensities of 0·3 to 0·5 watts/cm.^{2} were found to be the most effective in producing abnormalities. Accordingly a large number of embryos were fixed at several intervals after treatment at this intensity applied to eggs 2 hours after deposition; 80 per cent. of these embryos showed some kind of abnormal development. The abnormalities found in the serial sections are described here in developmental sequence.

(*a*) *Syncytial blastoderm stage (embryos fixed immediately after treatment).* In some embryos a vortex-like disturbance of the pole-cells alone was to be seen. More usually in the region of the disturbance there was a mixture of cytoplasm, yolk granules, pole-cell nuclei, and blastoderm nuclei (Plate 1, fig. 2). Frequently an irregular darkly stained mass was found in the abnormal region, probably as a result of fragmentation of yolk granules (Plate 2, fig. 9). The size of the abnormal region varied; this may have been due to changes in the mechanical strength of the blastoderm itself, which might increase with the number of nuclei included in it as the period of cell-wall formation approached.

When subsidiary disturbances had occurred in other regions of the egg the break in the blastoderm was accompanied by the movement of cytoplasm, yolk, and blastoderm nuclei to the surface of the embryo. With very small breaks in the blastoderm, only yolk granules moved to the egg surface. In a few embryos clefts in the cytoplasm perpendicular to the egg surface were formed (Plate 1, fig. 3). Nuclei lined up along these clefts which probably occurred as ruptures produced as a result of strain.

(*b*) *Cellular blastoderm, gastrulation, and histogenesis* ($3\frac{1}{2}$ *to* 10 *hours*). The abnormalities which occurred in gastrulation depended upon the extent and position of the original disturbed area. In undamaged regions the cells which formed were typical elongate blastoderm cells. In those embryos in which the damaged region was large no gastrulation movement of any kind occurred, although cellular proliferation and rarely some differentiation of cell types took place. Some embryos (e.g. Plate 1, fig. 4) gave indications of gastrular differentiation, such as the formation of the germ-band in undisturbed areas; later, invagination of the anterior midgut rudiment took place.

In other embryos at this stage more complete gastrulation movements occurred, but various abnormalities in the processes of gastrulation could be observed. The cephalic furrow did not form in some; in others it formed but was incomplete, or the orientation of the cleft was abnormal. In yet other embryos the cleft remained a conspicuous feature long after it ceased to be obvious in normal embryos of the same age. Abnormalities were also observed in the formation of the posterior midgut invagination. In those embryos in which the damage at the posterior pole was considerable, the posterior invagination did not form. The invagination formed in other embryos, but the elongation took place from the posterior pole or the ventral surface. In some embryos the posterior invagination formed and moved anteriorly but the pole-cells were not included within it; when this happened the shape of the posterior invagination

was affected, because the walls of the invagination were apposed throughout their length instead of assuming a sack-like shape. The germ-band was asymmetrical in those embryos for which the damaged region extended farther along one side than the other.

Later, the anterior midgut did not form in some cases or it formed on one side only. Formation of the stomodaeum was frequently affected; it usually failed to invaginate. In other embryos it formed but was abnormal in position. The presence of free yolk on the surface caused the shape of the embryo to be distorted.

By 6 hours, nuclei which were in or bordering on the abnormal region had become very large (Plate 1, fig. 4) and showed some clumping of chromatin. It is probable that these nuclei increased in size by endomitosis as no mitotic figures were found. The pole-cells remaining in abnormal positions retained, in some instances, their distinctive cytological characteristics for several hours.

In some embryos the only differentiation which was obvious was the formation of the primitive gut; in others there were present small cells similar to ganglion cells. Mitotic activity continued for some time in even the most abnormal embryos.

During histogenesis many of the abnormalities which became conspicuous later in development could already be discerned. Particularly obvious at this time were abnormalities in hypodermal differentiation and in the development of organs which originate from the superficial ectoderm.

Abnormalities were frequently observed in the orientation of ganglion cells and the neuroblast cells from which they were formed. Whereas in normal embryos the unequal division of the neuroblast takes place in a direction perpendicular to the ventral surface with the small cell which is to become a ganglion cell budding off at the end nearest the centre of the egg (Poulson, 1950), in treated embryos the neuroblast divisions may be in a plane parallel to the ventral surface and the relative positions of ganglion cell and neuroblast cell may vary.

(*c*) *Organogenesis* (12 *to* 20 *hours*). The embryos fixed in this group revealed the end results of treatment. The expression of abnormality varied, depending upon the position and size of the regions affected at the early stage. The abnormal embryos were classified into five groups.

(i) The first group of seven embryos showed little or no development after treatment. Six of these showed mitotic divisions and in a few instances the spindles appeared abnormal, although subsequent degeneration generally made detailed cytological examination impossible. In one embryo the nuclei were very large with clumped chromatin, but these were arranged round the periphery as are normal nuclei at the syncytial blastoderm stage. The abnormal nuclei resembled those described previously as having developed in regions directly affected by the treatment.

(ii) The second group of eleven embryos showed cellular proliferation with-

out differentiation into the various types of tissue characteristic of the embryo (Plate 1, fig. 5). The disturbed region was always very large in these embryos.

(iii) In another group of twenty embryos differentiation but no morphological organization had taken place. The shape of these embryos was usually reminiscent of gastrular form, but in some (Plate 1, fig. 6) the resemblance was obscured by the extent of the damaged regions. Differentiation of the nervous system into ganglion cells and fibres always occurred in these embryos although the fibres were not well organized and might be only slightly in evidence. Mesoderm was present and in some cases had differentiated into elongate cells; elongation of cells, however, was never followed by fusion, and no muscles were formed. The gut was represented in some cases but not in others; often the cells were clumped and disorganized, but tubular portions sometimes appeared, usually in the anterior end. These embryos were no doubt those in which early gastrulation processes had been very abnormal.

(iv) Twenty-seven embryos showed markedly abnormal organization, which was expressed in several different ways. In some embryos spatial relationships between organs were distorted; in some, certain organs and tissues showed abnormal differentiation; in others certain organs and tissues were incomplete or lacking. Various combinations of these abnormalities were also found (Plate 1, figs. 7–8; Plate 2, figs. 9–12).

A common abnormality was the differentiation of the posterior spiracles and the posterior spiracular atrium at the dorsal surface near the middle of the embryos, with the anus also in the dorsal midline (Plate 1, figs. 7 and 8). In one embryo (Plate 2, figs. 9 and 10) the posterior spiracles developed in the posterior region, but with the opening of the spiracles deep in the interior of the embryo. The posterior end of this embryo resembled a hollow ball one side of which had been pushed in by pressure applied with the thumbs. In this embryo the anus opened at the posterior end.

Abnormalities were frequently found in the formation of the pro-ventriculus as a result of disturbed spatial relationships between the oesophageal and gastric portions of the structure. The development of the two portions seems quite independent, for in embryos in which the oesophageal component was lacking, or was not in contact with the gastric portion, the gastric portion still differentiated and the peritrophic membrane was sometimes secreted.

Various abnormalities in the spatial relationships of the salivary glands have been noted. In one embryo (Plate 2, fig. 11) the glands were on the same side of the embryo; in another the distal end of one of the glands was directed towards the anterior rather than the posterior end as in normal embryos. The presence in the glands of a staining substance indicated that the secretory function of the glands was not impaired.

Involution of the head rarely took place, and the shape and position of the cephalopharyngeal apparatus and mouth hooks were abnormal in the three

embryos in which there had been some involution (Plate 1, fig. 8, and Plate 2, fig. 9).

The most spectacular of the abnormal spatial relationships was the formation in two embryos of the gonads at the anterior end near the region of the mouth parts (Plate 2, fig. 12).

Differentiation of organs and tissues was abnormal in many embryos. In only one of the embryos examined did normal gut differentiation occur; differentiation of the visceral musculature was also rudimentary. Clumping of muscle-cells was frequently observed. In one embryo (Plate 2, fig. 11) no organs or tissues derived from the mesoderm were formed, although undifferentiated mesoderm cells were present.

Some incomplete formation or absence of organs and tissues occurred in every embryo showing abnormal organogenesis. Some cases, such as the failure of the tracheal branches to unite, were purely mechanical in nature. Mechanical abnormalities also account for the absence of the posterior gut rudiment, foregut and stomodaeum, as well as absence of gonads. In almost half of the embryos in which organization was aberrant, the salivary glands were lacking; this was related to abnormalities in the differentiation of the superficial ectoderm.

The hypodermis was incomplete in all treated embryos and was totally lacking in one (Plate 2, fig. 12). Those portions of the embryo which develop from the frontal sac (which moves superficial ectoderm from the surface to the interior by invagination) were lacking in all but three embryos. In all embryos showing abnormal organogenesis, mesoderm and nervous tissue were present together with at least some portions of gut, although the latter might occasionally consist only of clumps of cells.

Segmentation was abnormal in all these embryos. This was in part due to the absence of hypodermis and the abnormal differentiation of muscles, but free yolk at the surface also resulted in exaggerated segmentation (Plate 1, fig. 7).

(v) A group of thirteen embryos showed slight abnormalities, the most frequent of which was the absence of gonads or slight abnormalities in segmentation. In a few cases there was abnormal distribution of yolk granules, either in tissues or in regions of the gut where yolk is not normally found. In one embryo some muscle-cells, which had undergone elongation and partial fusion, were completely enclosed by nervous system.

(vi) Twenty-seven of the treated embryos which were fixed at this age were apparently normal in all respects.

Cytological Abnormalities

Although some cytological abnormalities were produced by treatment at the syncytial blastoderm stage with ultrasonics of intensity 0·3 to 0·5 watts/cm.^{2} (see above), it was found during ancilliary experiments that cytological abnormalities were produced most frequently after treatment at a slightly earlier stage with ultrasonics of higher intensity. The normal cytology of the material is described

by Huettner (1933). Forty-nine eggs were fixed between 5 and 15 minutes after treatment 1 hour after deposition using ultrasonic intensities between 0·5 and 1·2 watts/cm.^{2} for 30 seconds; the permanent serial sections subsequently prepared were examined using an oil-immersion objective.

Nine eggs showed cleavage divisions with abnormalities of spindle and centriole which were probably caused by the streaming motion of cytoplasm and yolk during treatment. Thus instances were found of centrioles displaced from their interphase nuclei. One centriole was situated equidistant from two nuclei in late prophase; it formed the pole of two mono-polar spindles which were forming one in connexion with each set of chromosomes, there being no other centriole associated with either nucleus. A dipolar spindle with centrioles was found outside and slightly displaced from a nucleus whose nuclear membrane was intact (Plate 2, fig. 14). One end of a spindle at metaphase was found to be split into two parts each of which had a single centriole at a separate pole, while the opposite half of the spindle was normal: the spindle therefore appeared tripolar. Another metaphase spindle was found split in a direction perpendicular to the metaphase plate (Plate 2, fig. 15).

There were several instances of chromosomes displaced laterally at metaphase without, however, being completely separated from the spindle. In two eggs no chromatin at all could be found in the many cleavage divisions, which were recognized by the presence of apparently normal, but empty, spindles together with their centrioles and asters (Plate 2, fig. 16). The cytoplasm of these eggs had been disturbed by the treatment to the usual extent but appeared otherwise normal, with yolk granules intact; moreover the staining was unimpaired, as could be gauged from the deeply stained appearance of the condensed chromosomes of the polar bodies. It was most striking to note how in the majority of cases the spindles and asters of the mitotic figure protected the cleavage divisions from mechanical disturbance by streams of moving yolk and cytoplasm during treatment. Twenty eggs from this group showed cleavage divisions which were apparently quite normal in every respect, in spite of the fact that the contents of the egg had obviously been stirred round. Two eggs showed only normal mitotic figures, but these exhibited a definite loss of synchrony not attributable to a fixation gradient. Four eggs of the group were normal but of a much later stage of development, while thirteen others were too badly damaged to analyse.

Eight eggs were fixed 3 to 4 hours after ultrasonic treatment at intensities 0·5 to 1·2 watts/cm.^{2} applied during the late cleavage stage (i.e. 1 hour after deposition). One of these eggs was an abnormal gastrula, but the other seven showed nuclear proliferation with no sign of nuclear migration to form a syncytial blastoderm. Each of these seven eggs contained obviously polyploid nuclei and the polyploidy must be presumed to have developed subsequently to and as a result of the treatment. Large numbers of metaphase figures with polyploid chromosome sets were found (e.g. Plate 2, figs. 18 and 19); some of these had apparently normal spindles, centrioles, and asters, while in others the spindles and asters

were irregular and of indefinite polarity, and the centrioles were sometimes lacking. Groups of centrioles, each with its aster, were to be seen in the cytoplasm remote from any nearby nuclei. Enormous resting-stage nuclei of irregular shape were also present (Plate 2, fig. 13); one of these was 40μ long. One of these eggs was found to contain 8 diploid metaphase figures (including one with a split spindle), 9 polyploid metaphase figures with normal spindles and centrioles, and about 80 polyploid metaphase figures either with centrioles absent or with the spindle abnormal. There were also 3 normal diploid resting-stage nuclei in the same egg together with about 120 abnormally large resting-stage nuclei of a wide range of size and shape. In the cytoplasm about 190 free centrioles with asters were found.

DISCUSSION AND CONCLUSIONS

In many eggs which were treated with ultrasonics at preblastodermal stages, reconstitution of the ooplasmic contents followed and development continued normally. Pauli (1927) observed a similar reconstitution after centrifugation of *Calliphora* eggs before blastoderm formation; the stratification of the ooplasmic contents by centrifugation, however, is not similar to the redistribution of the egg contents as a result of ultrasonic treatment. Partial reorganization was never observed in eggs which had been subjected to ultrasonic treatment at this stage; either no reorganization followed and degeneration began, or embryos developed which were normal except for very slight abnormalities in a few cases.

It is possible that interference with mitosis is responsible for some of the early deaths in eggs treated with ultrasonics at preblastodermal stages. Howland (1941) frequently obtained abnormalities of spindle configuration and function after centrifuging early egg stages of *Drosophila*, and these were probably related in part to changes in the nature of the egg cytoplasm. Some evidence has been presented for similar cytoplasmic changes taking place in some eggs on treatment with ultrasonics, and it is possible that these caused some of the abnormalities in spindle function and led to the observed polyploidy. The displacement of centrioles and some cases of distortion of spindles in our experiments are, however, more readily attributable to direct mechanical effects caused by streams of heavy yolk granules moved by the action of the ultrasonic waves. It may be significant that the cytological abnormalities which Bessler (1952) obtained in gastrulae of newt by ultrasonic treatment were obtained in a yolky material. In *Drosophila* eggs the absence of cell membranes between the cleavage nuclei before blastoderm formation makes the streaming movements caused by the ultrasonic treatment all the more effective in producing cytological abnormalities; from this point of view therefore the material is more favourable than the meristematic tissues used by Selman (1952) for studying the cytological effects of ultrasonics. However, the chromosomes of the present material are not particularly favourable for the observation of induced abnormalities.

It is probably coincidence that the period of maximum lethality for ultrasonic

treatment and various other treatments such as X-radiation (Packard, 1926, 1935) and alpha-radiation (Hanson & Heys, 1933) is the syncytial blastoderm stage. It is not surprising that the nuclei are highly susceptible to the effects of radiation at this stage for they are undergoing rapid division near the outer surface of the egg. On the other hand, the lethal effects of ultrasonic treatment at this time seem only rarely to be related to nuclear abnormalities; more frequently they are caused by changes in the blastoderm which occur as a result of the rotation of the pole-cells, the formation of the vortex, and the concomitant displacement of the egg constituents.

None of the experimental methods which have been applied to insect eggs are comparable in their effects with those obtained with ultrasonic treatment. Centrifugation, which like ultrasonic treatment can result in a shift in the contents of the egg at early stages, is not able to cause a shift in the blastoderm once it has been established (Pauli, 1927; Howland, 1941). The other methods are dependent upon the removal of restricted regions of the egg, without altering the positions of nuclei. In eggs subjected to ultrasonic treatment, as a result of the progression of the blastodermal cells towards the region of disturbance, the spatial relationships within the blastoderm are altered. In addition to the change in position of nuclei which are supposedly 'determined' when they enter the peripheral cytoplasm, there is in all probability a movement of the innermost regions of the cytoplasm quite independent of nuclear movements, so that the cytoplasmic environment of nuclei may be substantially different to that in normal embryos. The abnormalities in gastrulation which occurred after treatment are easily explained by considering the mechanical difficulties arising as a result of the presence in the embryo of abnormal regions caused by disturbance at the time of treatment. Some abnormalities are the result of changes in position of blastodermal nuclei, so that the cells which formed the stomodaeum, for instance, were more ventral than in normal embryos. Failure of shortening during the middle period of embryogenesis sometimes caused the posterior tracheal regions and the anus to occupy very abnormal positions. Other morphogenetic movements occurring at the same stage, such as head involution and dorsal closure, were also affected.

The end results of treatment are of more interest. In some embryos considerable proliferation of cells took place after treatment, although there was no cellular differentiation. Pauli (1927) has described embryos which developed from eggs which had been centrifuged and in which there was multiplication of cells without differentiation; these all developed from eggs which had been centrifuged for long periods of time with the result that the ooplasmic contents became very disorganized. Sonnenblick (1940) and Sonnenblick & Henshaw (1941) found many embryos showing this phenomenon after one parent had been treated with X-rays; frequently part of the cytoplasm in these embryos remained non-cellular but stained deeply with basic dyes (cf. Plate 1, fig. 5). In the cases described by Pauli cytoplasmic factors were probably involved, and it is probable that the

underlying cause of the abnormality produced by ultrasonic treatments is similar. It is not impossible, however, that chromosomal factors may have caused the abnormality, as they most probably did in dominant lethals which were induced with X-rays by Sonnenblick (1940).

Differentiation of tissues without any organogenesis in embryos after ultrasonic treatment at the syncytial blastoderm stage seemed to be related to the failure of normal gastrulation. It would seem that even in mosaic eggs certain morphogenetic movements must take place before organogenesis occurs, even in regions such as the foregut and stomodaeum which develop in place after they form. It is also interesting to note that although the nervous system in these embryos showed typical differentiation into nerve-fibres and ganglion cells, no other evidence of differentiation of ectodermal tissues was found. Mesoderm and some gut cells were present, but their differentiation was slight.

The most interesting embryos, from the point of view of information about factors in differentiation and organization, were those in which a considerable degree of organization had occurred, but in which there were also many abnormalities.

The simplest kind of abnormality to explain was the distortion of spatial relationships within the embryo. The mechanical difficulties which prevent the shortening of the embryo result in the differentiation of the posterior spiracles in a position which they do not normally occupy at this stage. The movement of the whole blastoderm during treatment also leads to invagination of organ rudiments in abnormal positions. A shifting of blastoderm may also lead to abnormality in the position of the salivary glands which has been observed in some embryos (e.g. the embryo in Plate 2, fig. 11). Another factor of consequence is that the development of one organ in an abnormal position will force organs which form later into positions which they do not normally occupy.

Abnormalities in cellular differentiation are more difficult to explain satisfactorily. It is especially difficult to find the explanation of the high degree of involvement of the hypodermis, which was affected in every embryo in which organization had taken place. It is improbable that during treatment cells from which the superficial ectoderm will differentiate are preferentially destroyed; for the action of the ultrasonics is such that all cells in a particular region are involved. It is also highly unlikely that the superficial ectoderm develops entirely from cells at the posterior end. There seem to be at least two alternative explanations, neither of which can be proved because our knowledge of the processes of differentiation in mosaic eggs is so scanty. One possibility is that the development of the hypodermis depends on some special cytoplasmic factor or submicroscopic cellular structure which is particularly easily destroyed by the treatment. The other, which merits more discussion, is that the great sensitivity of the hypodermis is the consequence of a disturbance of some inductive relationship.

Poulson (1945) has suggested, on the basis of abnormal differentiation in

embryos deficient for the facet region of the X-chromosome, that there may be an inductive relation between the mesoderm and the ectoderm, the former acting as an inductor. His arguments are not very strong. If an inductive relationship of the kind proposed by Poulson does exist, it is not dependent upon differentiation of the mesodermal layer, for in one embryo (Plate 2, fig. 11) the mesodermal elements were completely disorganized although the hypodermis was better developed than in any other embryo studied.

More convincing evidence of inductive relationships between the two layers has been produced by the experimental work of Bock (1939, 1941) and Haget (1953*a*). Although this was performed on Neuroptera and Coleoptera, and not on Diptera, one may perhaps use it as a guide in interpretation. They find that the ectoderm (hypodermis) first exerts an inductive influence to which the mesoderm reacts. Later the mesoderm has an inductive influence on the development of the midgut (endoderm). Finally Haget in particular has emphasized the existence in beetles of an 'intra-dermal' induction process within the hypoderm, by which the development of the outlying parts is affected by their relation to the prothoracic region.

In seeking an explanation of the lack of differentiation of the hypodermis in the ultrasonic-treated embryos, it seems that one might appeal to this last type of relation, and suppose that the treatment had inhibited in some way an intradermal induction process which should normally occur within the ectoderm. It may be noted that the intradermal induction as described by Haget takes place between blastula formation and the end of gastrulation, so that one might expect a similar process in *Drosophila* to be strongly affected by ultrasonic treatment at the syncytial blastoderm stage.

In making this suggestion one is using processes found in one group of Insects, the Coleoptera, to throw light on phenomena occurring in another group, the Diptera. That the drawing of such parallels may be dangerous is suggested by another type of induction relation, that between the germ-cells and the mesodermal elements of the gonads. Haget (1953*b*) has shown that in Coleoptera the mesodermal part of the gonad develops even if the germ-cells are completely destroyed at the pole-cell stage. Aboim (1945) has claimed the same thing for *Drosophila* in which the pole-cells have been killed by ultra-violet light. However, in all the cases he illustrates there are germ-cells in the gonads, although they are dead and disintegrating. Since it is well known that dead tissues may exert evocating influences, his evidence cannot be taken as proving that the mesodermal gonad can differentiate in the absence of a stimulus from the germ-cells. In our material they certainly show no signs of such ability. The gonads are absent in many embryos in which the pole-cells were trapped in an abnormal region at the posterior end, so that it was impossible for them to be carried into the interior of the embryo. In some of these cases, where the extent of the damage at the posterior end was great, the cells which form the primordium of the posterior midgut were affected so that no invagination was formed by which

pole-cells could be carried to the interior. However, in these embryos the pole-cells were so surrounded by the abnormal tissue that their movement seemed unlikely in any case.

On the other hand, we find positive evidence for the ability of the germ-cells to induce gonad formation. In two embryos (one of which is shown in Plate 2, fig. 12) the gonads formed in the anterior region just behind the mouth parts. In both instances, although the germ-cells had migrated into regions of mesoderm with which they are not normally associated, there was clear differentiation of the gonad sheath and the interstitial elements of the gonads which are formed from mesodermal cells. This would seem to indicate that the fate of some mesodermal cells is not completely determined. The possibility that a great shift in cellular blastoderm had occurred during treatment cannot be ruled out entirely in the case of one of these embryos, for other organs which are normally found anteriorly were in the posterior region. The orientation (anterior and posterior) of this embryo could be determined by the position of the chorionic filaments: there is also a characteristic thickened region at the posterior end of the chorion which may be used as an additional guide to orientation. The possibility of an error in interpretation as to anterior and posterior ends of embryos is therefore completely excluded. In the other embryo, however, the other organs developed in positions which were quite normal; in fact it was this embryo in which the most normal spatial relationships of organs in the anterior end, including the cephalopharyngeal apparatus and the chitinized structures of the anterior region, were found.

The abnormalities in tracheal formation and salivary glands may be explained from abnormalities in the separation of cells of the superficial ectoderm, since both organs develop from cells of the superficial ectoderm which begin to invaginate at about the seventh hour of embryonic life. The failure of head structures to develop can also be related to abnormalities in ectodermal differentiation, because the frontal sac, from which the chitinized structures of the head are derived, first forms as an invagination of superficial ectodermal cells in the anterior end of the embryo.

The slight abnormalities in organogenesis are also easily understood. Abnormal distribution of yolk is responsible for some of the abnormalities in segmentation which have been observed, while slight abnormalities in muscle differentiation or apodemal attachment account for others. The presence of a small clump of muscle-cells in the nervous system of one embryo may be the result of the inclusion of determined cells within an area destined to become something else. The cells subsequently developed independently into muscle-cells, although they were completely surrounded by nervous tissue.

The results obtained in treating embryonic stages with ultrasonics seem to justify the further use of ultrasonics in experimental embryology. There are indications that further work along these lines might reveal valuable information concerning the possibility of the existence of inductive relationships in *Droso-*

phila embryos. It might also be possible to use ultrasonic treatment as a tool in solving the problem of the fate of the pole-cells in the embryo. It is known that some of the pole-cells form the gonads, but the fate of the others is unknown. It is also a matter for conjecture whether the pole-cells which enter the embryo before the formation of the ventral furrow form yolk-cells as suggested by Rabinowitz (1941), or form the gonads as Poulson (1950) has proposed. Poulson further suggests that the pole-cells which are moved to the interior in the posterior midgut invagination take part in the formation of the midgut only. Between the third and fourth hours of development the pole-cells lie in a shallow concavity formed by the cells of the posterior midgut anlagen. It might be possible, by treating embryos at this stage with ultrasonics, to move the pole-cells out of this concavity and prevent them from entering the embryo. If those pole-cells which have migrated previously are those which form the gonad, and the others are part of the midgut anlagen, as Poulson suggests, then the majority of embryos treated at this stage should have normal gonads, but the formation of the gut should be affected. Ultrasonic treatment of other insect embryos in which development is less determinate than *Drosophila* but in which determination is still a cytoplasmic function, such as in *Camponotus* which was studied and reviewed by Reith (1931), should give even more interesting developmental results.

SUMMARY

1. The use of ultrasonics as a tool for the experimental study of embryonic processes in *Drosophila* has been investigated.

2. It has been found that developmental changes can be induced by the application of ultrasonic waves at intensities sufficiently low to obviate the possibility of cavitation damage or chemical change.

3. The effects of ultrasonic waves of various intensities on different developmental stages has been studied by direct visual observation during treatment and in permanent preparations of sectioned material at subsequent developmental stages.

4. Treatment at preblastodermal stages was usually followed by readjustment and normal but delayed development; in other cases death ensued without further development.

5. Treatment of eggs in late cleavage stages with ultrasonic intensities of 0·5 to 1·2 watts/cm.^{2} for 30 seconds produced abnormalities of spindle, displaced centrioles, and induced polyploidy.

6. Treatment at the syncytial blastoderm stage, with ultrasonic intensities 0·3 to 0·5 watts/cm.^{2} for 30 seconds, resulted in the production of a high proportion of developmental abnormalities which were classified into five different categories: (*a*) death without further development; (*b*) proliferation of cells without differentiation; (*c*) differentiation without organization; (*d*) abnormal organization; and (*e*) slight abnormalities in organization.

7. The possible causes of the different types of abnormalities in embryonic development after treatment with ultrasonics are considered, and are discussed in relation to abnormalities in embryogenesis which have been produced in Dipteran embryos by other means, and in the light of the general morphogenetic situation existing in Diptera and certain other insect orders. Further applications of the method to developmental studies are suggested.

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EXPLANATION OF PLATES

Abbreviations:

A	anterior	DBN	displaced blastoderm nucleus	NUC	nucleus
AMG	anterior midgut	G	gut	OES	oesophagus
BLD	blastoderm	GB	germ-band	P	posterior
BN	blastoderm nucleus	GO	gonad	PC	pole-cell
BR	brain	HEC	head segment	PMUS	pharyngeal muscles
BT	bristle	HY	hypodermis	SLG	salivary gland
CEN	centriole	MG	midgut	SP	spiracle
CH	chorion	MP	Malpighian tubules	SPA	spiricular atrium
CHF	chorionic filament	MS	mesoderm	TR	trachea
CHT	chitin	MUS	muscle	VAC	vacuole
CLN	cleavage nucleus	NF	nerve-fibres	VM	vitelline membrane
CYT	cytoplasm	NS	nervous system	VNS	ventral nervous system
				YK	yolk

Magnification: In figs. 2, 10, and 13–19 inclusive the scale represents 10μ. In all other figures it represents 100μ.

Plate 1

Fig. 1. Longitudinal section of an egg treated with ultrasonics at 0·3 watts/cm.^{2} 1 hour after deposition, and fixed immediately after treatment, showing the curved outline of the disturbed regions. Cleavage nuclei are present. Note the absence of yolk granules at the periphery of the disturbed areas.

Fig. 2. Enlargement of the posterior region of an egg treated with ultrasonics at the syncytial blastoderm stage, and fixed immediately, showing the point of rupture of the syncytial blastoderm, and a typical disturbed area. Note the irregular dark mass, probably formed of fragmented yolk globules.

Fig. 3. Anterior region of embryo treated with ultrasonics at 0·3 watts/cm.^{2} during the syncytial blastoderm stage and fixed immediately. A disturbed region has formed at the anterior end, and yolk has flowed out of a small gap on the dorsal surface, distorting the shape of the embryo. Nuclei and cytoplasm in the disturbed region are abnormally distributed.

Fig. 4. Longitudinal section of embryo treated with ultrasonics at 0·3 watts/cm.^{2} during the syncytial blastoderm stage. Age at time of fixation, 6 hours. Only rudimentary gastrulation has taken place. The abnormal nuclei which form in the disturbed areas are clearly shown. Note the separation of yolk and cytoplasm in the abnormal region.

Fig. 5. Semi-longitudinal section of embryo treated with ultrasonics at 0·5 watts/cm.^{2} during the syncytial blastoderm stage. Age at fixation, 19½ hours. The anterior end is a cellular mass without any differentiation. The abnormal region occupies almost the entire posterior half.

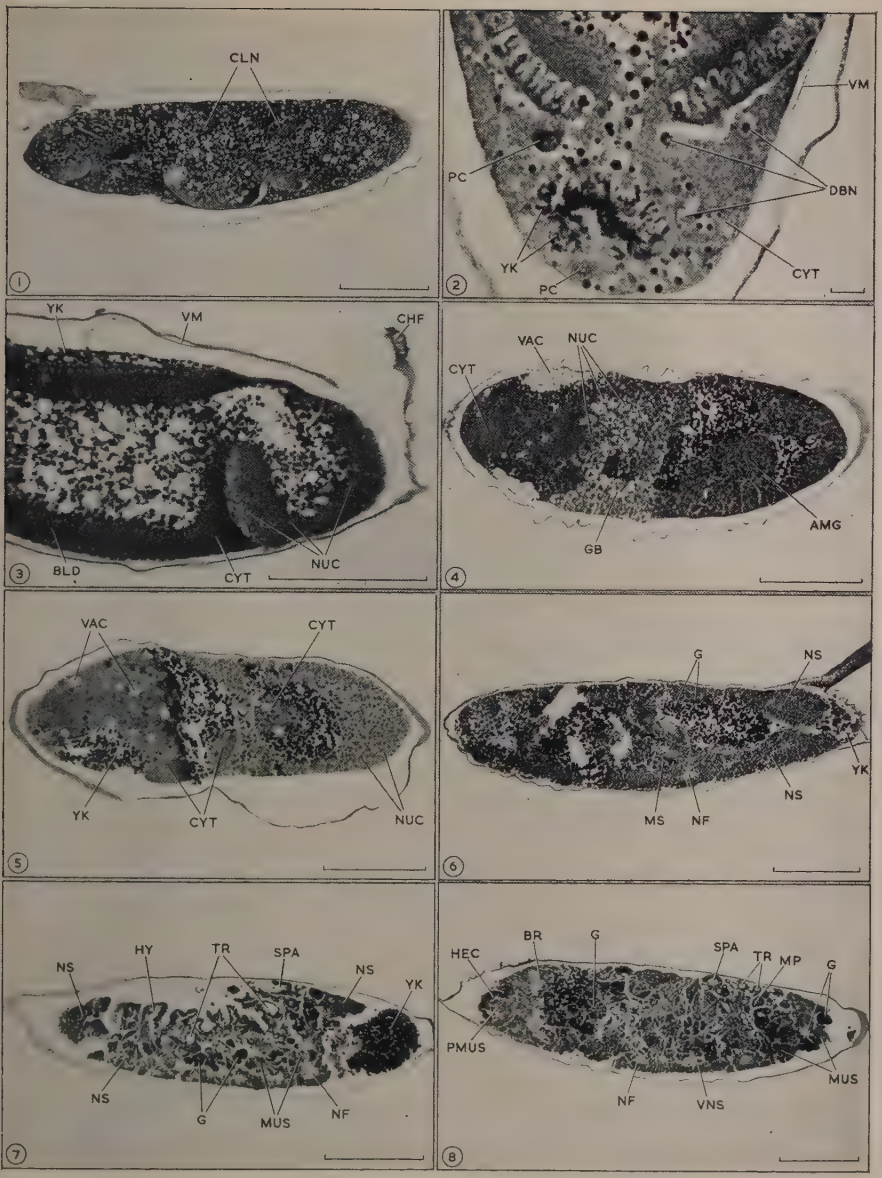
Fig. 6. Longitudinal section of embryo treated with ultrasonics at 1·2 watts/cm.^{2} during the syncytial blastoderm stage. Age at fixation, 19 hours. Differentiation of tissues, but no organization, has taken place.

Fig. 7. Longitudinal section of embryo treated with ultrasonics at 1·2 watts/cm.^{2} during the syncytial blastoderm stage. Age at fixation, 19 hours. Note the exaggeration in segmentation at the dorsal surface. No hypodermis is present ventrally. The atrium of the posterior spiracle is on the mid-dorsal surface, which indicates that shortening of the germ-band has not occurred. The central region is a mixture of cells of the midgut and muscle-cells.

Fig. 8. Median longitudinal section of the embryo shown in fig. 7. The mixture of muscle-cells, tracheal fragments, and gut cells in the central region is conspicuous. Involution of the head is abnormal, but there has been some organization of pharyngeal musculature.

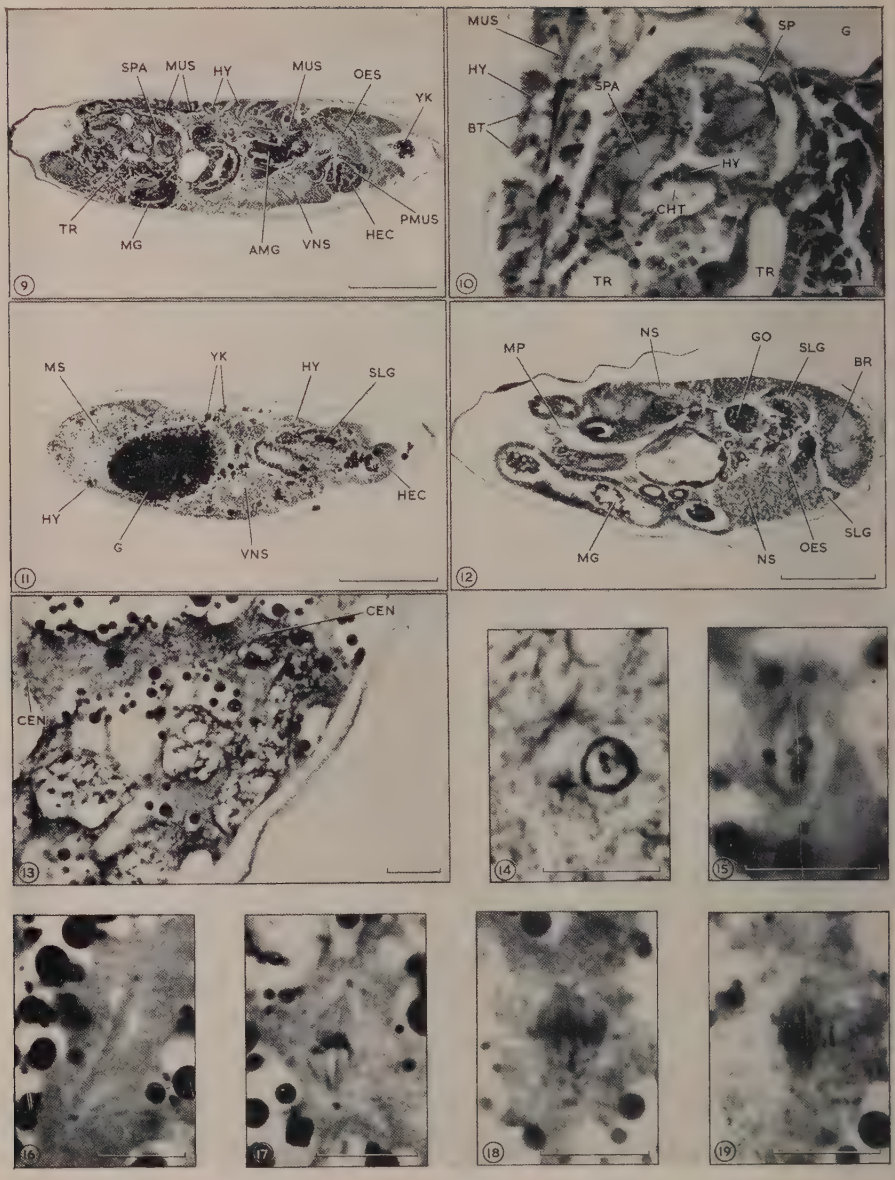
Plate 2

Fig. 9. Median longitudinal section of embryo treated with ultrasonics at 0·3 watts/cm.^{2} during the syncytial blastoderm stage. Age at fixation, 20 hours. Segmentation is present only in the dorsal posterior region. The anterior dorsal region and the ventral region are composed almost



S. J. COUNCE & G. G. SELMAN

Plate 1



S. J. COUNCE & G. G. SELMAN

Plate 2

entirely of nervous tissue. The head segment has formed too far posteriorly, but the pharyngeal musculature is quite well organized. At the posterior end the embryo appears pushed in like a hollow ball to which local pressure has been applied. The posterior spiracles and spiricular atria may be seen in the interior.

FIG. 10. Enlargement of the posterior region of the embryo shown in fig. 9, showing the structure of the posterior spiracles. The structure of the hypodermis is also shown.

FIG. 11. Longitudinal section of embryo treated with ultrasonics at 1·2 watts/cm.² during the syncytial blastoderm stage. Age at fixation, 18 hours. The mesoderm is disorganized. No differentiation of the gut from a primitive state has occurred, and the oesophagus and proventriculus (not shown) are very abnormal. Differentiation of the hypodermis is good, with the exception of the dorsal surface where it is lacking (failure of dorsal closure?). The salivary glands are displaced, and lie on the same side of the embryo, one more dorsally than the other. The presence of staining material in the lumen indicates that the functional activity of the glands has not been impaired. No mouth parts have formed, but the hypodermis in the anterior region is thickened. No true segments have formed.

FIG. 12. Semi-longitudinal section of embryo treated with ultrasonics at 1·2 watts/cm.² during the syncytial blastoderm stage. Age at fixation, 19 hours. The hypodermis is completely lacking. The gonad has formed in the anterior region. Differentiation of the gut and of the visceral musculature is almost normal.

FIG. 13. Enormous resting-stage nuclei in an egg treated with ultrasonics at 1·2 watts/cm.², 1¼ hours after deposition, and fixed 4 hours later. Note the centrioles.

FIG. 14. Spindle displaced to the side of a resting-stage nucleus, in an egg treated with ultrasonics at 1·2 watts/cm.², 20 minutes after collection, and fixed 5 minutes later.

FIG. 15. A metaphase spindle split along its length in an egg treated with ultrasonics at 1·2 watts/cm.², 1 hour after deposition, and fixed 5 minutes later.

FIG. 16. Showing a spindle with centrioles and an absence of chromosomes, in an egg treated with ultrasonics at 1·2 watts/cm.², 1 hour after deposition, and fixed 5 minutes later.

FIG. 17. A normal diploid metaphase figure in an egg treated with ultrasonics at 0·5 watts/cm.², 1¼ hours after deposition, and fixed 5 minutes later.

FIG. 18. A polyploid metaphase figure (contrast with fig. 17), in an egg treated with ultrasonics at 1·2 watts/cm.², 1¼ hours after deposition, and fixed 4 hours later.

FIG. 19. A polyploid metaphase figure, in an egg treated with ultrasonics at 1·2 watts/cm.², 1¼ hours after deposition, and fixed 4 hours later.

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Morphogenetic Effects of a Heat Shock on the Eggs of *Limnaea stagnalis*

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WITH TWO PLATES

INTRODUCTION

A TREATMENT of the eggs of *Limnaea stagnalis* with lithium chloride at early stages of development causes characteristic aberrations of development (Raven, 1942). In some of the eggs gastrulation is disturbed, which leads to the production of vesicular or dumb-bell-shaped exogastrulae. Other embryos show a normal gastrulation, but at later stages various malformations, especially of the head region, become visible. Partly they belong to the cyclocephalic series: synophthalmia, cyclopia, anophthalmia, acephaly. Besides these, other malformations, such as unilateral reduction of eyes and tentacles, reduplication of one or both eyes, or malformations of the mouth parts, may be produced.

A study of forty-two embryos with cyclocephalic malformations produced by lithium treatment (Raven, 1949) showed that these malformations are due to a suppression of mediodorsal parts of the head. In normal development the head organs (cerebral ganglia, eyes, and tentacles) develop from two fields of small ectoderm cells, situated on either side of the midline, the cerebral plates. At later stages these plates are represented by two lateral areas covered by a high epithelium, the tentacle fields. They are separated by a median band of 7 large ciliated cells, the apical plate; this extends from the upper margin of the mouth to the head vesicle, a transverse girdle of 12 big swollen cells behind the cerebral plates. Laterally and ventrally the latter are surrounded by the large ciliated cells of the prototroch or velum.

Cell-lineage studies in *Planorbis* and *Physa* (Holmes, 1900; Wierzejski, 1905) have shown that the posterior four cells of the apical plate are derived from the cells immediately surrounding the original animal pole of the egg. In the cyclocephalic monsters produced by lithium treatment in *Limnaea*, in all cases, the differentiation of this part of the apical plate had been suppressed. This was attended, in a large majority of the cases, by a fusion of the tentacle fields of both

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sides. Moreover the cerebral commissure was very often shortened, and the cerebral ganglia of the left and right side had fused in the middle to a common mass. Finally, the differentiation of the head vesicle, the velum, and the anterior part of the apical plate had often been suppressed. Together these aberrations form a syndrome that may be considered as being quite typical for the class of cyclocephalic malformations in snails.

It was concluded from these observations that lithium can act in *Limnaea* on a gradient field with its high point at the animal pole of the egg. It has a depressing influence on the maximum of the gradient field, by virtue of which the cells in this region are forced to differentiate in a way characteristic of lower levels of the gradient in normal embryos, so that tentacle field and eye instead of apical plate develop from mediodorsal cells of the head.

This conclusion was corroborated by a study of eighty-four exogastrulae produced by lithium action (Raven, 1952). In these exogastrulae various tissues exhibit a certain degree of differentiation. Small- and big-celled endoderm, stomodaeum and oesophagus cells, mesenchyme and larval kidney, small- and big-celled ectoderm may be distinguished. As regards the ectodermal hemisphere, an essential diversity in the topographical relationships of big- and small-celled ectoderm was found. In some of the cases (type I) two fields of small-celled ectoderm are surrounded by an 8-shaped area of big ectoderm cells. This arrangement agrees with that in normal embryos, the fields of small ectoderm representing the cerebral plates. In other cases (type II) these plates have fused to one area of small-celled ectoderm, lying near the animal pole and surrounded on all sides by a girdle of big ectoderm cells. This corresponds to the situation found in cyclocephalic monsters. Finally, in some of the exogastrulae (type III) no small-celled ectoderm at all can be found in the animal hemisphere, which is entirely composed of big ectoderm cells. Hence these three types represent a progressively increasing series of suppression of animal differentiations, and give further support to the hypothesis that one of the effects of lithium treatment consists in the weakening of a gradient field with high point at the animal pole. In addition to this, lithium has an injurious action on the material of the vegetative hemisphere, expressing itself in an inhibition both of the morphogenetic movements and of the histological differentiation of these cells.

Brachet (1949) observed that a heat shock in Amphibia caused aberrations of development superficially resembling those obtained by lithium treatment. In *Limnaea* the effects of a heat shock have been studied by Visschedijk (1953). Heating the eggs to 37° C. for from 15 minutes to 3 hours at the uncleaved, 4-cell or 24-cell stage causes, besides mortality, the production of a certain number of exogastrulae and head malformations. The mortality and the number of malformations rise with increasing duration of the heat treatment. The susceptibility of the eggs to heat shows a progressive decrease from the uncleaved stage onwards.

The head malformations obtained by this treatment belonged, according

to Visschedijk, partly to the cyclocephalic series (synophthalmia, cyclopia, anophthalmia); partly they were of another kind. As this statement was based on a study of living embryos only, it appeared necessary to check it by a study, both of the exogastrulae and the head malformations obtained by a heat shock, in sections. In particular, this investigation should answer the question whether there are any indications of a depression of the animal gradient field by this treatment, comparable to that caused by lithium.

MATERIAL AND METHODS

The egg capsules of an egg-mass were freed of the surrounding jelly at the 2-cell stage (stage 6–7 according to Raven, 1946). They were then transferred to a small glass tube with 3 ml. of tapwater and placed in a water-bath for the required time. This water-bath was kept constant at a temperature of $36{\cdot}8 \pm 0{\cdot}4°$ C. For the production of a maximum number of head malformations a treatment of $1\frac{1}{2}$ hours was used; when especially exogastrulae were to be formed, 3 hours' heating was applied. After treatment the egg capsules were laid out on an agar bottom and cultured at 25° C. From the third day after treatment the exogastrulae formed were isolated; they were cultured for another 24 hours, then removed from the capsules and fixed. After 6 or 7 days, but often considerably later, the surviving embryos reached the 'hippo'-stage (cf. Raven, 1949). Those showing head malformations were now drawn, decapsulated, and fixed.

Both the exogastrulae and the embryos with head malformations were fixed in Bouin's fluid; they were embedded in paraffin, and sectioned at $7\frac{1}{2}\mu$. The direction of sectioning of the 'hippo'-stage embryos was transverse. The slides were stained with iron haematoxylin and erythrosin.

RESULTS

I. *Exogastrulae*

A great number of exogastrulae were obtained with the above treatment. As a rule they were approximately spherical, often very big, vesicular structures. Superficially they resembled in every respect the exogastrulae produced with lithium treatment.

On microscopical study of the sections, however, it appeared that these vesicles differed considerably from lithium exogastrulae. The latter are, as a rule, rather uniform in structure, and different cell types can easily be distinguished, which are distributed over the surface of the exogastrula according to a characteristic pattern. Although the histological differentiation of the cells is often not altogether normal, and is arrested at a certain stage, still the various cell types can easily be related to the tissues of a normal embryo, and their pattern reflects the derivation of the latter from the regions of the blastula (Raven, 1952). On the contrary, in the exogastrulae produced by a heat shock the distinction between the tissues is much more difficult. Apparently the differentiation of the cells

has taken an abnormal course from the beginning so that the result is highly atypical. As a rule it is even impossible to distinguish between an ectodermal and endodermal hemisphere. Mostly a large part of the wall of these vesicles is occupied by big cells, between which one, two, or more groups of small cells are found. The latter either form a more or less regular unistratified epithelium or they are heaped together in irregular masses, bulging either outward or inward into the cavity of the exogastrula. They greatly resemble the small-celled ectoderm of lithium exogastrulae. The big cells, on the other hand, correspond in their differentiation neither to the big-celled ectoderm nor to the big-celled endoderm of lithium exogastrulae. As a rule they combine the characteristics of both. Their nuclei are mostly large and clear and possess rather big nucleoli. Their cytoplasm is often rather pale and may contain clear watery vacuoles; on the other hand, very often one or more huge albumen vacuoles are found in it, too. Often all big cells on the surface of the exogastrula are of the same 'mixed' type. If, however, cells with clear cytoplasm (resembling ectoderm) and those with albumen vacuoles (resembling big-celled endoderm) are found side by side, their topographic distribution is, apparently, entirely arbitrary. Graphical reconstructions of thirty-two of these exogastrulae have been made, in which it was attempted to indicate as far as possible the distribution of various cell types. They yielded, however, nothing useful; no regularity in the distribution of various cell types could be established. It is clear, therefore, that development in these exogastrulae produced by a heat shock is much more heavily disturbed than in lithium exogastrulae.

Besides true exogastrulae, in which the invagination of the archenteron had been entirely suppressed, a number of embryos with incomplete invagination of the archenteron have been studied. As a rule the invaginated material occupied only a small part of the cavity. A stomodaeum had often differentiated in these cases; in a few instances a shell gland had been formed. In general, however, their differentiation was hardly better than in the exogastrulae, so that they likewise yielded no information with respect to the problem under consideration.

II. *Head malformations*

Of a total of about 1,500 eggs, subjected to a heat shock for $1\frac{1}{2}$ hours, 38 embryos with head malformations were found; this is about $2\frac{1}{2}$ per cent. Thirty-one of them have been studied in sections.

The head malformations found in these embryos belong to various classes. In 7 embryos one of the eyes is reduced; 1 embryo is completely anophthalmic. Fifteen embryos are triophthalmic; among them two types must be distinguished. In 9 cases a supernumerary eye has been formed on one side of the head; in 6 embryos there is, between the two normal eyes, a supernumerary eye in the dorsal midline. One further embryo is tetrophthalmic, possessing both a lateral and a median supernumerary eye. Finally, there are 7 embryos exhibiting

various displacements of head organs, especially of the mouth parts; in 3 of them a considerable dislocation of the nervous ganglia has taken place.

a. Reduction of one eye

In 7 embryos one of the eyes has been reduced; curiously, in all 7 cases it is the right eye. In 6 of them no trace of an eye has been found on the right side (monophthalmia); in 1 embryo the right eye is rudimentary (microphthalmia asymmetrica). In all cases the left eye is normally developed. The right lateral tentacle may be somewhat smaller than the left; in some of the cases, however, no difference between the tentacles of both sides can be observed.

In general, no other deviations of normal development have occurred in these embryos. The tentacle fields of both sides are separated by a well-developed apical plate (Plate 1, fig. A). In two cases (Nos. 20 and 21) this apical plate is partly reduplicated (cf. below, p. 147). The nervous system is entirely normal; the cerebral ganglia are situated at their normal place; no shortening of the cerebral commissure has occurred. The only further abnormality concerns the velum, which was present in 3 cases, absent in 3, while in 1 case it is uncertain whether a velum is present.

b. Anophthalmia

In one embryo both eyes are lacking; one pigmented cell in the left tentacle may represent the only vestige of an eye left. The epithelium of the left tentacle field is strongly folded, and its differentiation is rather poor. The velum is lacking. Otherwise no abnormalities can be found. The apical plate and head vesicle are well developed. The nervous system is entirely normal; no shortening of the cerebral commissure has taken place.

c. Supernumerary lateral eye

As stated above, 10 embryos (9 triophthalmic and 1 tetrophthalmic) possess a supernumerary lateral eye. In 5 cases it is situated on the right side, in the other 5 on the left. The supernumerary eyes show various dispositions. In 3 cases there is only a beginning reduplication of one of the eyes, which is somewhat elongated and possesses two cavities, in each of which a lens has been formed. The reduplicated eye lies at its normal place in the base of a lateral tentacle. Two embryos have on one side two clearly separated but contiguous eyes, both lying in the tentacle base. In 2 further cases the two eyes of one side lie at some distance from each other (Plate 1, fig. B); one is situated in its normal position, whereas the other lies more dorsally. Finally, in 3 embryos the normal and the supernumerary eye lie wide apart; in one of these cases the supernumerary eye is found in a supernumerary lateral tentacle in front of the normal one; in the other two it is situated dorsoposteriorly with respect to the normal eye, in the neighbourhood of the cerebral commissure (Plate 2, fig. G). Apparently these cases form a series in which the tendency to reduplication

becomes more and more manifest, beginning with the formation of a single, but too large eye invagination with a tendency to bipartition at further development, and ending with the development of two eye centres wide apart in a single tentacle field. As noted above, in one of the latter cases a reduplication of the lateral tentacle has also taken place. Curiously, however, the reduplication tendency of the cerebral plate has not, as a rule, influenced the development of the cerebral ganglion. Only in one case, with beginning splitting of the eye (No. 34, cf. below), the cerebral ganglion of that side has perhaps a supernumerary lobe; in all other cases no difference between the cerebral ganglia of both sides has been observed.

In the two embryos in which the supernumerary eye is situated far dorso-posteriorly near the cerebral commissure, the posterior part of the apical plate is poorly developed; in places it seems to be lacking altogether (Plate 2, fig. G). Two other embryos (Nos. 10 and 34, cf. below) show a reduplication of this part of the apical plate. In the other embryos the apical plate is normal. The head vesicle is normal in 6 cases, uncertain in 4. The velum, however, is lacking in all 10 cases.

No other deviations of development have occurred in this group. The nervous system is entirely normal (apart from the supernumerary cerebral lobe mentioned above). No shortening of the cerebral commissure or fusion of the tentacle fields has taken place.

d. Supernumerary median eye

A supernumerary median eye is found in 7 embryos. In 5 of them this is accompanied by a reduplication of the posterior part of the apical plate. Four further embryos show the latter phenomenon alone. Together these 11 embryos form a coherent group of related malformations, which, however, partly overlaps the previous ones, 2 embryos (Nos. 10 and 34) also possessing a reduplicated lateral eye, whereas 2 other embryos (Nos. 20 and 21) show a reduction of the right eye.

We will first give some examples of this syndrome of malformations.

No. 34. The embryo has a reduplicated eye in the base of the right lateral tentacle. The right cerebral ganglion has perhaps a supernumerary lobe. The other organs of the head are normal.

The apical plate begins above the mouth and extends posteriorly as a well-developed median band of ciliated cells. At the level of the reduplicated right eye it shifts gradually to the left of the midline. At the same time, a second row of apical plate cells appears on the right side; both apical plates lie nearly symmetrically with respect to the median plane (Plate 1, fig. D). They are separated by a plate of small ectoderm cells, resembling the epithelium of the tentacle field. The reduplicated apical plate may be followed for five sections of $7\frac{1}{2}\mu$, then both rows merge with the head vesicle. This is well developed, but the velum is lacking.

A second embryo (No. 15) shows a similar partial reduplication of the posterior part of the apical plate.

No. 38. The apical plate begins above the mouth as a single row of cells, which, however, after two sections already bifurcates. Between the two rows of ciliated apical plate cells there is a median area of small-celled ectoderm having the character of tentacle field epithelium. This area bulges outward, forming a more or less conical prominence. It extends over nine sections backwards, its epithelium gradually becoming thinner. In its posterior part a supernumerary median eye is situated beneath the epithelium. It is still at an early stage of differentiation, but has already a well-developed lens (Plate 1, fig. E). Finally, the two rows of apical plate cells bordering the median tentacle field merge with the well-developed head vesicle, which forms the posterior boundary of this tentacle field.

No further malformations of head organs are present. The lateral tentacles and eyes and the nervous system are entirely normal. It must especially be emphasized that there is no accessory cerebral ganglion beneath the median tentacle field. The velum is lacking, however.

No. 5. This embryo resembles the previous one in many respects. It has also a triangular median tentacle field, bordered by a reduplicated posterior part of the apical plate laterally and the head vesicle posteriorly, and containing a supernumerary median eye (Plate 2, fig. F). But the embryo is much older, and the dorsal part of the head has been considerably stretched. The unpaired anterior part of the apical plate, which extends over sixteen sections in this case, has therefore strongly decreased in transverse diameter. In many sections it only forms a small notch in the midline between the left and right tentacle fields, and in some sections it is not even visible at all. Presumably, however, this is a secondary phenomenon due to stretching and has nothing to do with a true suppression of apical plate differentiation.

As in the previous case, no other malformations of head organs are present, with the exception of the velum which is completely lacking.

No. 10. This is the tetrophthalmic embryo mentioned above. In the first place, it has a reduplicated right eye. But besides this there is a median tentacle field also containing an eye. The apical plate, beginning at the upper margin of the mouth, after four sections bifurcates into two rows of apical plate cells, which extend through nine sections until they merge into the strongly developed head vesicle. The triangular area of small ectoderm cells, bordered by the bifurcated apical plate laterally and the head vesicle posteriorly, has the character of tentacle field. In its anterior part it bulges strongly outward, forming a well-developed supernumerary median tentacle (Plate 1, fig. C). Posteriorly it has given rise to a supernumerary median eye. This has shifted, however, in a posterior direction during development, so that it has come to lie beneath the greatly swollen head vesicle cells (Plate 2, fig. H).

Again, the other head organs are normal, except the velum, which is lacking.

As mentioned above, 11 embryos may be put in this group. In 2 of them the posterior part of the apical plate is doubled by a second row of apical plate cells, but the two rows do not come together, only one of them extending anteriorly towards the mouth. In these cases no supernumerary median eye has been formed. Seven embryos have an accessory dorsomedian tentacle field, bounded by the two halves of the reduplicated apical plate laterally, and by the head vesicle posteriorly. In 5 of these cases this field has formed a supernumerary median eye; moreover, in some cases it bulges outward, forming a well-developed accessory tentacle. Finally, 2 embryos have a supernumerary median eye, but show no distinct reduplication of the apical plate; the differentiation of the posterior part of the apical plate is rather unclear in these cases.

In general, no other malformations of head organs are present. It must be especially emphasized that the accessory median tentacle field has not given rise to a supernumerary cerebral ganglion. Only in one case may some supernumerary ganglion cells be present in this region. The only further abnormality is the absence of the velum; only in 3 of these 11 embryos is a velum present; in all other cases it is lacking.

e. Dislocation of the nervous ganglia

In 3 embryos a curious dislocation of the nervous ganglia has taken place. This is linked up with a deviation in the development of the mouth parts, which will be described in greater detail in another paper. The invagination of the stomodaeum has been disturbed in these cases; either no invagination has taken place at all, or the already invaginated stomodaeum has evaginated again at a later stage. As a consequence of this, no buccal cavity and pharynx have been formed; the cells lining these cavities in normal embryos have remained at the surface and have extended considerably, now covering a large protruding area of the head. Into this area the oesophagus debouches as a narrow tube. At some distance from the oesophagus the radular sac has formed as an isolated invagination.

As a rule the unfolding of the everted stomodaeum has taken place asymmetrically, so that it covers either the right or the left side of the head. In consequence, the other head organs have been pushed towards the opposite side. The fact that, with the eversion of the stomodaeum, a pull has apparently been exerted on the midgut, so that this has penetrated into the head region, tends to make the topography of the head organs still more abnormal.

In the three embryos mentioned this development has given rise to the following situation (Plate 2, fig. J): the pedal ganglia, united by the pedal commissure, are situated in their normal location in the foot. One of the pedal ganglia is connected by the cerebropedal connective with the cerebral ganglion of that side, which is situated above it. However, the whole dorsal part of the head, with the cerebral ganglia, tentacle fields, and eyes, has rotated through 90° to one side, so that the other cerebral ganglion lies above the first, and at a great

distance from the pedal ganglion of its side; no cerebropedal connective has formed on this side. Curiously, in two of these cases normal statocysts are present on both sides, lying against the pedal ganglia. This proves that the development of the statocysts is not dependent on normal topographical relations between the pedal and cerebral ganglia. In the third case a statocyst is only found on the side in which there is a cerebropedal connective.

DISCUSSION

1. In accordance with Visschedijk's (1953) results, it appeared that a heat shock at 37° C. for 3 hours, applied at the 2- to 4-cell stage in *Limnaea*, produces a great number of exogastrulae. These exogastrulae differ considerably, however, from those caused by lithium treatment, in showing a much more serious disturbance of development, leading to the appearance of very atypical differentiations. Whereas the lithium ion has a localized effect, influencing the animal gradient field, on the one hand, and the material of the vegetative hemisphere, on the other (Raven, 1952), the effect of a heat shock seems to be much more generalized. Presumably the intrinsic development of the cells is forced in an abnormal direction from an early stage; the disturbance of gastrulation is only one of the secondary effects of this primary deviation. In as far as local differences in cell type were found, they showed no regularity of pattern which could in any way be compared with the topography in a normal embryo. These exogastrulae were useless therefore in connexion with the problem as to whether a heat shock has a local action on the animal gradient field; if it has, its effect is obscured in the exogastrulae by its general injurious action on cell differentiation. Better results might perhaps be obtained with exogastrulae produced by a heat shock of shorter duration. The experiments are continuing in this direction.

2. According to Visschedijk, the head malformations caused by a heat shock belong partly to the cyclocephalic series (synophthalmia, cyclopia, anophthalmia). In our experiments neither synophthalmic nor cyclopic embryos have been found. As a matter of fact, one anophthalmic embryo was produced; but, as we shall see, this does not belong to the cyclocephalic series either, but links up with the monophthalmic embryos showing a local suppression of eye development.

The cyclocephalic embryos produced by lithium show the following characteristic syndrome of malformations: (1) absence of the posterior part of the apical plate, (2) fusion of the tentacle fields of both sides, attended with median displacement of eyes and lateral tentacles, (3) shortening of the cerebral commissure, with fusion of the left and right cerebral ganglion to a common mass (Raven, 1949).

In none of the 31 embryos studied in this investigation has this syndrome been found. As a matter of fact, in 2 cases the posterior part of the apical plate has not been found in the sections; in 5 other cases its differentiation is more or less uncertain or poor. However, in none of these cases is there a distinct fusion of the left and right tentacle field, a displacement of eyes or lateral tentacles, a median

fusion of the cerebral ganglia, or a shortening of the cerebral commissure. We may conclude, therefore, that *no typical cyclocephalic malformations have been produced by a heat shock.*

Our results, therefore, contradict the statement of Visschedijk mentioned above. We have been able to re-examine some of the embryos with head malformations obtained by Visschedijk in his experiments, and preserved by him in alcohol. In many of these embryos no eyes could be observed at all. Some of them may have been anophthalmic; in other ones the still faintly pigmented eye rudiments may have become invisible at fixation by the increased opacity of the tissues. Among the embryos treated by a heat shock at the 4-cell stage we further found 6 cases with reduction of the right eye, 1 with reduction of the left eye, 1 with reduplicated left eye, and 1 with a supernumerary median eye. In the group of head malformations obtained by a heat shock at the 24-cell stage, in addition to the anophthalmic ones there were 5 embryos with suppression of the right eye, 4 with reduction of the left eye, 4 with reduplicated right eye, and 2 which might with some hesitation be classed as synophthalmic. Apart from the latter rather doubtful cases, and the possibility that among the embryos in which now no eyes are visible there may be synophthalmic or cyclopic ones, it appears that the head malformations obtained by Visschedijk in general agree with those found in the present investigation.

As mentioned above, the production of the characteristic cyclocephalic malformations by lithium in *Limnaea* has been explained by assuming that lithium has a depressing influence on the animal gradient field. Apparently the effects of a heat shock do not agree with those of lithium in this respect. We may conclude, therefore, that a *heat shock*, at least at the 2- to 4-cell stage, *does not primarily act by depressing the animal gradient field in the same way as lithium may do.*

3. The morphogenetic effects of a heat shock on the structure of 'hippo-stage' embryos may be arranged in the following classes: (1) local eye reductions, (2) lateral reduplications, (3) median reduplications, (4) displacements of various head organs.

The first group consists of those embryos in which either one or both eyes are rudimentary (microphthalmia) or lacking (monophthalmia asymmetrica, anophthalmia). This suppression of eye development is not attended with a shifting of the eye anlage. As a rule it is the only deviation of development present, the other organs of the head being entirely normal; only the lateral tentacle of the same side may be somewhat reduced in size.

This malformation is not a typical heat-shock effect, for similar monophthalmic and microphthalmic embryos may arise after lithium treatment (Raven, 1942, 1949) or centrifugation (Raven & Beenakkers, 1955). Apparently eye development is easily suppressed by adverse influences on development.

A peculiarity of eye reduction following a heat shock at the 2- to 4-cell stage is the fact that this reduction affects preferentially the right side; against 7 embryos

with reduction of the right eye, no case of left eye reduction was found in our material. This is corroborated by Visschedijk's cases mentioned above: 6 reductions of the right eye against 1 with left reduction. The probability that such a proportion of 13 : 1 would arise by chance is very small (less than 1 per cent.); apparently, therefore, the susceptibility of the egg to such effects of a heat shock as lead to eye reduction is different on either side of the animal pole at the 4-cell stage. There seems to be no such difference at the 24-cell stage, as the proportion between left and right eye reductions in Visschedijk's embryos treated at that stage was 4 : 5. It is further remarkable that after lithium treatment the great majority of eye and tentacle reductions concern the *left* side (Raven, 1942). Together these facts suggest that *the left-right asymmetry at early cleavage stages in* Limnaea *is attended with differences in physiological conditions between both sides.*

4. The 'lateral reduplications' concern 10 embryos, exhibiting a reduplication of one of the eyes, ranging from the formation of two cavities in one elongated eye vesicle to the presence of two complete eyes lying a great distance apart, but still in one tentacle field. The 10 cases are equally divided between both sides of the head. In one case the eye reduplication is attended with the presence of a supernumerary lateral tentacle, both eyes lying at the base of a different tentacle. Apart from one rather doubtful case, the cerebral ganglion of the reduplicated side is normal. We may conclude, therefore, that the tendency to reduplication in most cases remains restricted to the formation of two eye-forming centres in a single tentacle field.

The eye reduplications represent no typical heat-shock effect. They may both occur after lithium treatment (Raven, 1942, 1949) and after centrifugation of the eggs (Raven & Beenakkers, 1955). However, in lithium embryos eye reduplication is mostly attended with a medial displacement of the eyes and other abnormalities, belonging to the syndrome of cyclocephalic malformations (Raven, 1949).

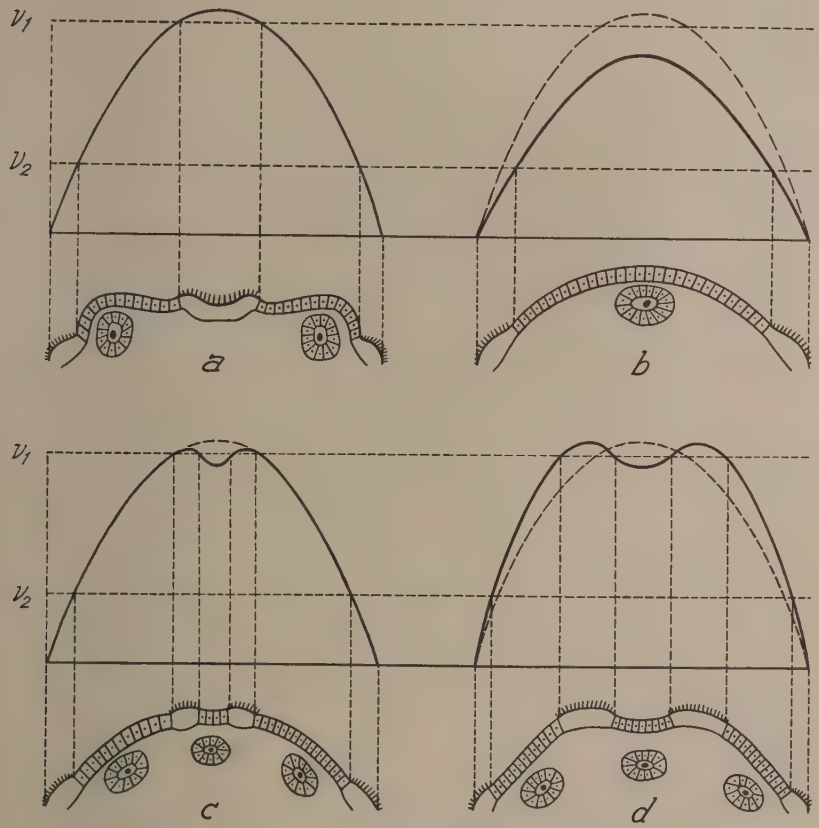
5. The median reduplications represent the most characteristic heat-shock effect, in so far as no similar malformations have been met with till now after other treatments. In their most typical form they consist of the following abnormalities: (1) The apical plate, beginning at the upper margin of the mouth as a median belt of cells, after a longer or shorter unpaired stretch, bifurcates; the two halves then diverge and continue separately towards the head vesicle. (2) The more or less triangular area, bounded by the two halves of the apical plate and the head vesicle, has the character of a tentacle field. It has formed an eye and sometimes a tentacle.

This syndrome of malformations, in its complete form, has been found in 5 out of 31 embryos. Six further embryos apparently belong to the same class, but are less typical, as part of the syndrome is lacking.

At first sight it may seem that these median reduplications can be considered together with the lateral reduplications from the same point of view. In both

cases a heat shock leads to a partial reduplication of head organs; in both cases it involves the eyes and gives rise to triophthalmic embryos.

However, further consideration shows that more can be said about the mechanism of the median reduplications. This is due to the fact that the structure



TEXT-FIG. 1. Diagrammatic representation of the effect of the animal gradient field on pattern of head organs in *Limnaea*. *a*. Normal embryo. *b*. Cyclocephalic embryo after depression of gradient field. *c–d*. Reduplication of apical plate after local depression (*c*), or strengthening (*d*) of gradient field.

which is reduplicated in these embryos, the posterior part of the apical plate, is of special importance in head development. As we have seen, it is derived from the most animal part of the egg. Our lithium experiments have led us to the view that it represents the high end of an animal gradient field (Raven, 1949). In other words: *the application of a heat shock has led in these embryos to a splitting of the apex of a gradient*. This is a remarkable phenomenon which deserves further consideration.

According to our hypothesis, the differentiation of the cells of the ectodermal hemisphere in *Limnaea* takes place under the influence of a gradient field. When we pass in a transverse direction from left to right along the cells of the blastula, the value of the factor underlying this gradient field first rises from the equator to a maximum at the animal pole, then decreases again towards the equator on the other side. We may presume that in the region where the intensity of the field surpasses a certain value v_1, the cells will differentiate into apical plate. Below v_1, but above a value v_2 of the field factor, differentiation to tentacle field (= cerebral plate) will take place. Finally, where the intensity lies below v_2, the cells will develop into velum. In this way the arrangement of structures in a transverse section of a normal embryo may be explained (Text-fig. 1*a*).

The action of lithium, which leads to the development of cyclocephalic embryos, consists, according to this view, in a depression of the gradient field as a whole. Accordingly the value v_1 will nowhere be reached, and the differentiation of apical plate is suppressed. The cerebral plates of left and right sides fuse in the midline, and two eyes, on either side of the median plane (synophthalmia), or a single median eye (cyclopia), are formed (Text-fig. 1*b*).

When we apply this line of reasoning to the present case it appears that a splitting of the apical plate, as observed in our embryos, may be explained in two different ways.

In the first place, such a splitting might be caused by a very circumscribed local depression of the gradient field at the animal pole. The intensity of the field in this area will be reduced to a value below v_1, and tentacle field epithelium will be formed here. On either side, however, in a small area the field factor rises above v_1, so that two strips of apical plate cells are formed (Text-fig. 1*c*).

On the other hand, however, the reduplication of the apical plate might be due, not to a depression, but to an intensification of the field. The following line of argument has led us to this conclusion.

It has often been remarked (e.g. de Vries, 1926) that in man and the vertebrates, starting from the normal structure of the facial region, a continuous series of malformations can be drawn up, exhibiting on the one hand increasing reduction of median structures (synophthalmia bilentica, synophthalmia unilentica, cyclopia synophthalmia, cyclopia perfecta, microphthalmia mediana, anophthalmia mediana); and showing, on the other hand, increasing enlargement, then reduplication, of median head-structures (diprosopus diophthalmus, triophthalmus and tetrophthalmus). In other words, the normal face forms the middle term of a series leading from anophthalmia mediana to diprosopus tetrophthalmus. As we now know, the cyclocephalic malformations are the result of a deficiency of the inductive mechanism. It is an obvious supposition that the diprosopus series, being the reverse of the cyclocephalic series, is due to an excess of inductive factors. In other words: *a surplus of determining factors does not lead to an unlimited increase in size of the corresponding organs, but to their reduplication.*

A similar line of thought has recently been developed by Dalcq (1947) with respect to the normal bilateral symmetry of head organs.

> It is only a quantitative relation which determines whether or not the olfactory and the optic apparatus will attain a symmetrical arrangement. . . . The symmetrical arrangement is linked with a certain degree of concentration of the inductive substances. . . . The general interpretation of these facts appears to be that there exists, for each organ, a size limitation. . . . When the number of elements exceeds the limit compatible with an organ unit, a rearrangement intervenes, and symmetry is acquired. . . . It is very probable that spontaneous double monsters are due to an excess of the precursors for dorso-marginal materials, so that two separate morphogenetic fields have to be formed.

If we accept this point of view, for the moment, it must be remarked that such a symmetrical arrangement of organs, either in normal development or in the case of reduplication, involves their separation by structures of a different type, therefore corresponding to another (presumably as a rule a lower) level of the determining factors.

Although the above-mentioned examples concern cases of embryonic induction, we might assume that a similar mechanism holds for those cases where determination is controlled by gradient fields. We may explain, therefore, the median reduplications obtained in our heat-shock experiments by an enhancement of the field factors, which automatically leads to a reduplication of the structures corresponding to the high end of the gradient, and their separation by structures corresponding to a lower level (Text-fig. 1*d*).

We will now try to decide which of the two explanations, that of Text-fig. 1*c* or 1*d*, fits the facts. A clue for answering this question is given by the quantitative relations of the structure corresponding to the highest field level: the apical plate. If the splitting of the apical plate is due to its local suppression (Text-fig. 1*c*), we may expect that the number of cells in its remaining parts will be less than normal. If, on the other hand, it is caused by an excess of field intensity (Text-fig. 1*d*), the number of apical plate cells will be equal to or larger than the normal number.

We have tried, therefore, to count the number of apical plate cells in our embryos with partially reduplicated apical plate. This is no easy task. Since the cell boundaries between the apical plate cells are only rarely visible with sufficient clarity, we counted the nuclei. They are large, clear (cf. Plate 1, fig. D; Plate 2, fig. F), and often more or less polymorphic, so that they mostly extend over more than one section. It is, however, often impossible to determine with certainty whether nuclear slices found in successive serial sections belong to the same or to different nuclei. Therefore we determined in each case the minimum number of certainly different nuceli present, and the maximum number on the assumption that every nuclear slice visible in the sections represents a different nucleus. The actual number must lie somewhere between both values. Table 1 gives the result of these counts in seven of the embryos in question (in two

embryos with apical plate reduplication the histological preservation did not permit accurate counting).

TABLE 1

Minimum and maximum numbers of nuclei in different parts of the apical plate

No.	*Unpaired part*	*Right part*	*Left part*	*Total*
5	2–3	2–4	1–2	5–9
10	3–7	1	1–3	5–11
15	5–7	2–5	4–8	11–20
21	6–7	1–2	—	7–9
23	—	5–11	4–8	9–19
34	4–10	1–2	2–6	7–18
38	5–6	2–5	2–5	9–16

If we compare the numbers found with the normal number (7) of apical plate cells, it is apparent that in three of these embryos (Nos. 15, 23, and 38) an unquestionable increase in the number of apical plate cells has taken place. In two further embryos (Nos. 21 and 34) this number is either equal to or larger than that of a normal embryo. With respect to two embryos (Nos. 5 and 10) nothing definite can be said, but it is probable that the number of apical plate cells is at least equal to normal.

We may conclude, therefore, that the splitting of the apical plate is not attended with a decrease in the number of apical plate cells, but in at least some of the cases with an increase. This pleads in favour of the view that *the median reduplications after heat-shock treatment are caused by a strengthening of the animal gradient field.*

Further circumstantial evidence for this view may be gained from the behaviour of the velum. Our lithium experiments have shown that lithium treatment does not cause a displacement of the ecto-endodermal boundary (Raven, 1952). We have concluded from this fact that the animal gradient field only controls the differentiation of the cells of the ectodermal hemisphere. Now the diagram of Text-fig. 1*d* shows clearly that under these circumstances a general elevation of the strength of the field will lead to a reduction of the structures corresponding to the lowest field levels, i.e. the velum. As a matter of fact, we have seen that in 8 out of 11 embryos with median reduplication the velum was lacking. Although it is possible that there are subsidiary causes for this reduction of the velum (as a matter of fact, a suppression of the velum has also been found in some of the cyclocephalic embryos, although one would here rather expect an enlargement of the velum, according to Text-fig. 1*b*), it is anyhow in good agreement with the point of view developed above.

6. We have emphasized above that, apart from one doubtful case in which some supernumerary ganglion cells were observed, the accessory median cerebral plate did not give rise to a cerebral ganglion. This does not agree with the

situation found in head malformations obtained by centrifuging (Raven & Beenakkers, 1955), in which a supernumerary cerebral plate, as a rule, in addition to an eye, formed also a cerebral ganglion.

In order to explain this difference, we must take into account the fact that the eyes, in normal development, develop from the dorsalmost part of the cerebral plates (Raven, 1952). Presumably, therefore, among the organs deriving from the cerebral plates, the eyes represent the highest gradient level, whereas the cerebral ganglia correspond to a lower level. The rearrangement of the gradient field leading to cranial reduplication brings about a relative depression in the area intermediate between the two new apices. Here the field value sinks below v_1, and differentiation to cerebral plate takes place (Text-fig. 1*d*). In view of the small size of this median cerebral plate, and its position between the two apices, it is easily understood that it will only give rise to the structure belonging to its highest level, i.e. eye.

7. We may now return to the lateral reduplications, in order to see if anything more can be said about their causation in the light of the above considerations.

In the first place it must be remembered that in all ten embryos with lateral reduplication the velum was lacking. In view of what has been said above, this may indicate that in these cases also an increase in the strength of the animal gradient field has taken place. Apparently this concerns especially the lower levels of the field, whereas no great changes have occurred near the field apex. This rise of lower field levels will bring about an increase in the extension of the cerebral plate area at the expense of the velum. But, as we have seen above, an excess of determining factors in a certain area may bring about a rearrangement of field factors leading to spontaneous reduplication. This may have taken place during the process of segregation of the organs within the cerebral plate. It is highly significant, in this context, that it is the eyes which show this reduplication; as we have seen, they presumably represent the organs of highest level within the cerebral plate area. *The lateral eye reduplication may, therefore, again be considered as a splitting of a (secondary) field apex.* Slight asymmetries in the configuration of the animal gradient field may be responsible for the fact that this occurs, as a rule, on one side only.

8. The dislocation of head organs, especially of the nervous ganglia, met with in these embryos, is no specific heat-shock effect. It is due, presumably, to a primary disturbance in the development of the stomodaeum, which may occur after various treatments of the eggs. These cases are of some interest, since they permit conclusions on the determinative interrelationships between various organs. From the embryos described in this paper we may deduce, for instance, that the differentiation of cerebral ganglion, pedal ganglion, and statocyst does not depend on the presence of a normal cerebropedal connective on that side of the body. We will return to these questions in a subsequent paper.

SUMMARY

1. Eggs of *Limnae stagnalis* were subjected to a heat shock (37° C.) for 1½ or 3 hours at the 2- to 4-cell stage. Both exogastrulae and head malformations were produced.

2. The exogastrulae produced by a heat shock differ considerably from those caused by lithium treatment in showing a much more serious disturbance of development, leading to the appearance of greatly atypical differentiations.

3. No typical cyclocephalic malformations were produced by this treatment. Apparently a heat shock does not primarily act by depressing the animal gradient field.

4. Local eye reductions were produced preferentially on the right side. Presumably the left-right asymmetry at early cleavage stages in *Limnaea* is attended with differences in physiological conditions between both sides.

5. Lateral eye reduplications, leading to the formation of two eyes in a single tentacle field, occurred on both sides.

6. The most characteristic heat-shock effect consists in a reduplication of the posterior part of the apical plate, with the formation of a median tentacle field containing an eye, and sometimes a tentacle.

7. The splitting of the apical plate is not attended with a decrease in the number of apical plate cells, but in at least some of the cases with an increase.

8. It is concluded that these median reduplications are caused by a strengthening of the animal gradient field, leading to the splitting of the apex of the gradient.

9. It is possible that the lateral eye reduplications are due to a splitting of a secondary field apex.

10. Dislocations of head organs, especially of the nervous ganglia, encountered in the treated embryos, show that the differentiation of cerebral ganglion, pedal ganglion, and statocyst does not depend on the presence of a normal cerebropedal connective on that side of the body.

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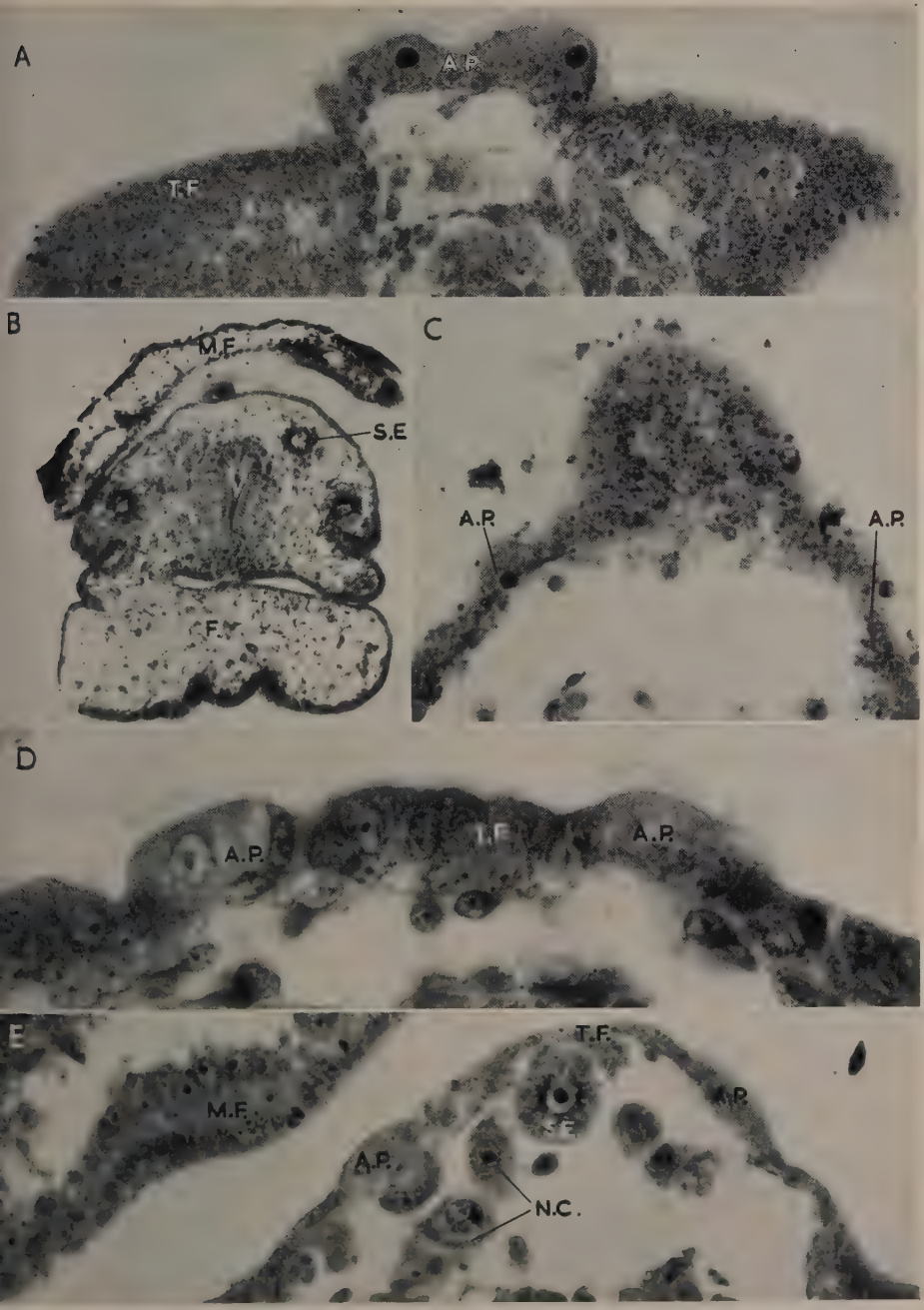

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Plate 1

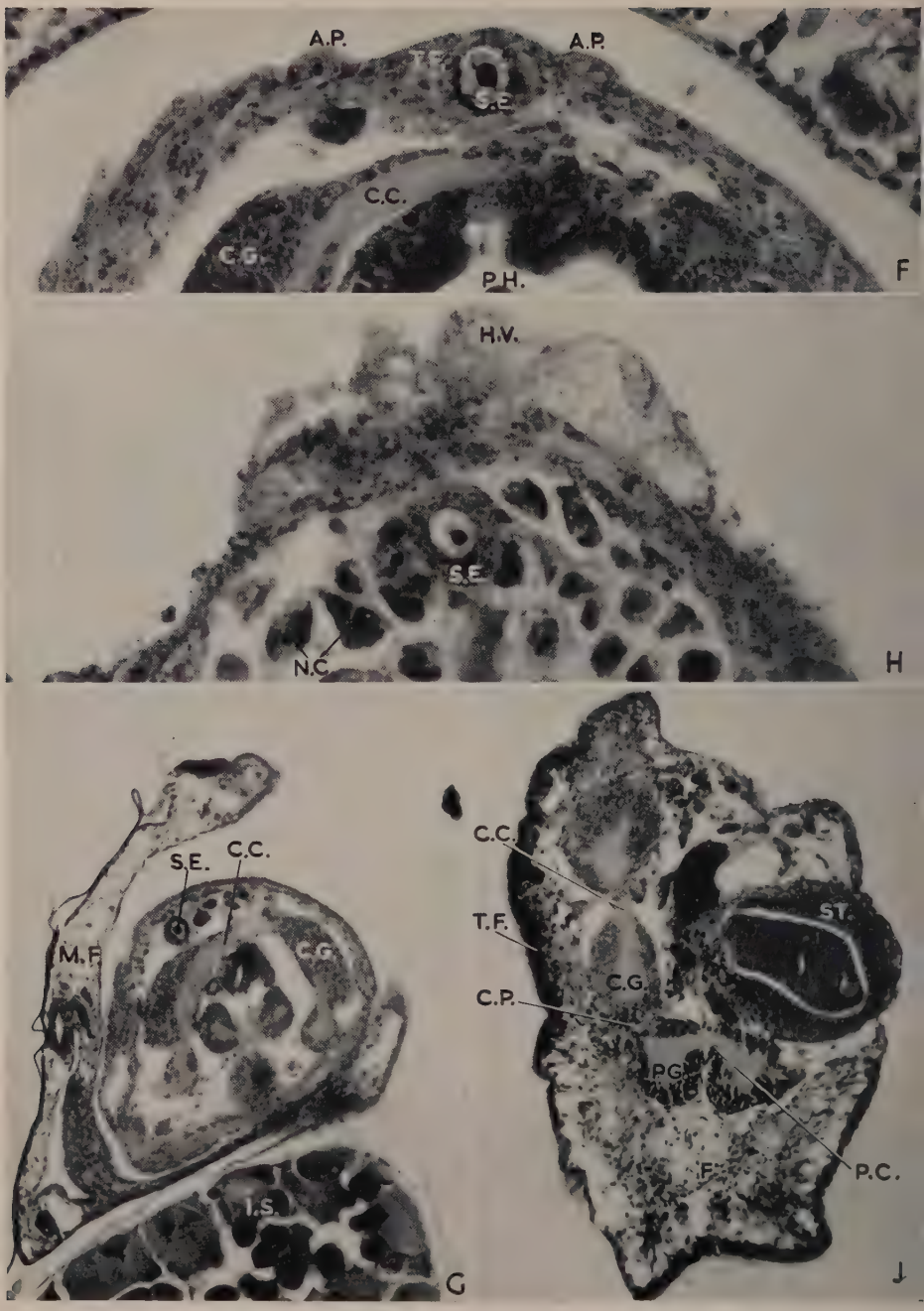

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Plate 2

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EXPLANATION OF PLATES

Abbreviations: A.P. apical plate; C.C. cerebral commissure; C.G. cerebral ganglion; C.P. cerebropedal connective; F. foot; H.V. head vesicle; I.S. intestinal sac; M.F. mantle fold; N.C. nuchal cells; P.C. pedal commissure; P.G. pedal ganglion; PH. pharynx; S.E. supernumerary eye; ST. stomach; T.F. tentacle field.

PLATE 1

FIG. A. Embryo No. 21. Tentacle fields of both sides separated by apical plate. The dark dots in the apical plate are albumen vacuoles. N.B. Some sections further posteriorly a bifurcation of the apical plate was found in this case. Magn. ×600.

FIG. B. Embryo No. 24. Transverse section of the head. Supernumerary left eye dorsal to normal eye. Magn. ×167.

FIG. C. Embryo No. 10. Supernumerary tentacle in median tentacle field, bordered by the two halves of the reduplicated apical plate. Magn. ×600.

FIG. D. Embryo No. 34. Median tentacle field between two rows of apical plate cells. Magn. ×1,200.

FIG. E. Embryo No. 38. Supernumerary eye in median tentacle field, bordered by the two halves of the reduplicated apical plate. Magn. ×600.

PLATE 2

FIG. F. Embryo No. 5. Supernumerary eye in median tentacle field, bordered by the two halves of the reduplicated apical plate. Magn. ×600.

FIG. G. Embryo No. 29. Frontal section. Supernumerary right eye near cerebral commissure. Magn. ×167.

FIG. H. Embryo No. 10. Supernumerary median eye beneath swollen head vesicle cells. Magn. ×600.

FIG. J. Embryo No. 30. Dislocation of head organs. The stomach has penetrated into the head region, the cerebral ganglia and tentacle fields have rotated through 90° towards the right side. Magn. ×167.

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Chimaerische Zahnanlagen aus Triton-Schmelzorgan und Bombinator-Papille

Mit Beobachtungen über die Entwicklung von Kiemenzähnchen und Mundsinnesknospen in den Triton-Larven[1]

von GERHART WAGNER[2]

Aus dem Zoologischen Institut der Universität Bern

MIT DREI TAFELN

Herrn Professor FRITZ BALTZER *zum 70. Geburtstag gewidmet*

I. EINLEITUNG

Die *xenoplastische Transplantation*, d. h. der Gewebeaustausch zwischen Vetretern verschiedener Gattungen, Familien oder Ordnungen, ist von der Spemann'schen Schule als sehr leitungsfähige Methode für die Erforschung der embryonalen Entwicklungsvorgänge erkannt und eingeführt worden. Zwischen Anuren (*Rana*) und Urodelen (*Triton*) gelang der Versuch erstmals Geinitz (1925) mit der Transplantation von Organisatormaterial, dann insbesondere Schotté mit der Transplantation von Epidermis (Spemann & Schotté, 1932). Er ist seither von zahlreichen Autoren auch mit vielen andern Organanlagen durchgeführt worden. Mehr und mehr berührten die Experimente nebst entwicklungsmechanischen auch stammesgeschichtliche Probleme. Dies ist besonders dann der Fall, wenn sich die transplantierten Anlagen bei den beiden Partnern stark divergent entwickeln, wie z. B. die Mundorgane von Anuren und Urodelen: Die Urodelen bilden schon im frühen Larvenstadium im Ober- und Unterkiefer echte Zähne, während dies bei Anuren erst zur Zeit der Metamorphose und nur im Oberkiefer geschieht.

In einer 1947 und 1948 ausgeführten Arbeit erhielten wir durch orthotope Transplantation von Kopfneuralleiste zwischen Bombinator und Triton in den Triton-Wirten öfters chimaerische Zahnanlagen mit einer Papille aus Bombinator-Mesektoderm und einem Schmelzorgan aus Triton-Epithel (Wagner, 1949). Diese Erscheinung war damals nur ein Nebenergebnis der Untersuchungen an chimaerischen Kopfskeletten und konnte, da die Experimente nicht speziell daraufhin angelegt waren, nicht mit der wünschenswerten Gründlichkeit analysiert werden. So blieben zahlreiche Fragen, deren Klärung im

[1] Ausgeführt mit Hilfe des Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung.

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Bereich des Möglichen schien, unbeantwortet, so z. B. die Frage, wie weit sich die chimärischen Zahnanlagen zu entwickeln vermögen. Aus beruflichen Gründen war der Verfasser in den folgenden Jahren verhindert, das Problem neu und auf breiterer Basis in Angriff zu nehmen. Erst im Sommer 1953 wurde dies durch ein Stipendium des 'Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung' ermöglicht. Dieser großzügigen Institution sei hier der wärmste Dank ausgesprochen. Die Herren Prof. F. Baltzer und Prof. F. E. Lehmann vom Zoologischen Institut der Universität Bern, in dem die Arbeit durchgeführt wurde, förderten diese durch großes Interesse und vielseitige Hilfe. Ihnen möchte ich an dieser Stelle ganz besonders danken.

II. TECHNISCHES

Zwischen jungen Neurulae von *Triton alpestris* und *Bombinator pachypus* wurde das Stück der Kopfneuralleiste ausgetauscht, in dem die Anlagen für den Trabecular-, Mandibular- und den Hyoidbogen enthalten sind. Das vorderste, stärker verdickte Stück der Neuralleiste und die präsumptive Kiemenregion

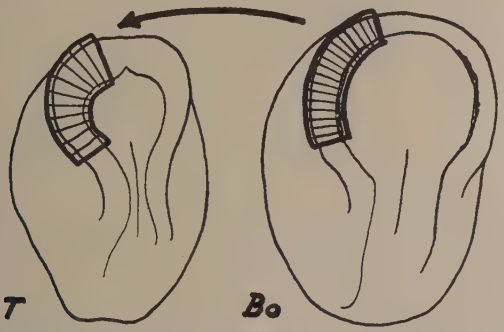

Abb. 1. *Operationsskizze.* Das ausgetauschte Neuralleistenstück enthält das Material des praesumptiven Trabecular-, Mandibular, und Hyoidbogens.

wurde möglichst wenig angetastet (Abb. 1). Wir betrachten im folgenden nur die Kombination Triton-Wirt mit Bombinator-Neuralleiste, da nur sie für die Bildung chimaerischer Zahnanlagen in Frage kommt (vgl. Wagner, 1949, S. 553).

Das Bombinator-Neuralleistenmaterial wandert in den Triton-Larven auf den durch die entodermalen Schlundtaschen gewiesenen normalen Wegen aus und bildet die drei genannten Visceralbogen in der für Bombinator typischen Gestalt (l.c. S. 531–9). Damit ist auch die Bedingung für die Entstehung chimaerischer Zahnanlagen gegeben.

Was die technischen Einzelheiten der Operation und Zucht anbetrifft, verweisen wir auf Baltzer (1941), Andres (1949), und Wagner (1949). Eine Verbesserung in der Zucht der Chimaeren wurde, dem Rat von Dr. H. E. Lehman, Chapel Hill, U.S.A., folgend, dadurch erzielt, dass dem Agar der Zuchtschalen 2 prozent Sulfathiazol beigemengt wurde, wodurch Infektionsgefahr und

Mortalität ganz wesentlich herabgesetzt wurden. Die Zuchttemperatur betrug 17–18°. Die in Bouin fixierten Tiere wurden durchwegs quer geschnitten. Dadurch erreichten wir, daß normale chimaerische Zahnanlagen viel häufiger längs getroffen wurden als in den Frontalschnitten der Arbeit von 1949. Gefärbt wurden die Schnitte nach Mallory (Romeis, 1948, § 1489), wodurch eine bessere Färbung des Dentins erzielt wurde. Nachteilig gegenüber der Kernfärbung mit Boraxkarmin war dabei der geringere Unterschied in der Färbbarkeit der Bombinator und Triton-Kerne. Die Schnitte wurden wieder nach Petry (1942) in Cyclon-Lack übergeführt, eine Methode, die für das Auswerten großer Schnittserien ganz wesentliche Vorteile bietet.

Die Unterscheidung der Bombinator- und Triton-Zellen ist in den Schnitten mit Hilfe von drei Kriterien im allgemeinen mit Sicherheit möglich: die Kerne von Triton sind größer, besitzen größere Nucleolen und färben sich stärker als die Bombinator-Kerne.

In der Stadienbezeichnung der Triton-Wirte halten wir uns an die Normentafel von Glaesner (1925). Das Alter geben wir in Tagen nach der Operation an. (Im Operationsstadium waren die Triton-Wirte 4 Tage, die Bombinator-Spender 2 Tage alt.)

III. ZAHNENTWICKLUNG UND ZAHNSUBSTANZEN BEI NORMALEN AMPHIBIEN

A. *Übersicht über die Literatur*

In der klassischen vergleichenden Anatomie galt es als feststehende Tatsache, daß alle Mund- und Hautzähne der Wirbeltiere aus den Hartsubstanzen Schmelz und Dentin bestehen, wobei der Schmelz als eine Bildung des ektodermalen 'Schmelzorgans', das Dentin als Produkt der mesodermalen Zahnpapille aufgefaßt wurde. Die Keimblattheorie hielt strikte daran fest, daß die Schmelzorgane, aus den Ameloblasten bestehend, eine spezifisch ektodermale, die Zahnpapillen aus Odontoblasten eine spezifisch mesodermale Differenzierung seien. In neuerer Zeit hat sich aber das Dogma der Keimblattspezifität gerade hier tiefgreifende Korrekturen gefallen lassen müssen.

Zunächst wurde es durch die Arbeiten von Platt (1897), Landacre (1921), Adams (1924), Raven (1931), Holtfreter (1935*a*), Sellman (1946), De Beer (1947), Wagner (1949) zur unumstößlichen Gewißheit, daß die Zahnpapillen der Neuralleiste entstammen, also ursprünglich ektodermaler Herkunft sind. Damit hätte man sich allenfalls noch abfinden können, nachdem einmal die Neuralleiste als zweiter Lieferant von Mesoderm (des Ekto-Mesoderms oder Mesektoderms) anerkannt war. Schon unglaubwürdiger erschien die Tatsache, daß bei Urodelen auch die den Zahnfeldern zugeordneten Knochen (die 'Zahnknochen', Hertwig (1906)), mit denen die Zähne nach ihrer Entstehung verwachsen, diesem Ekto-Mesoderm entstammen (Sellman, 1946; Wagner, 1949). Daß endlich die Schmelzorgane teils ektodermaler, teils entodermaler Herkunft sein

sollten, ging gegen alle Keimblattlehre. Und doch hat ein so guter Beobachter wie Goette schon 1875 diese Tatsache mit Sicherheit erkannt und ausgesprochen:

> ... Aber bei den Teleostiern, deren Darmblatt ebenfalls bis zum Lippenrande reicht und dort einfach mit der Oberhaut verschmilzt, sind alle Zähne ebenso gewiß Erzeugnisse der Darmblattschleimhaut wie wenigstens die Gaumenzähne der Batrachier. (Fußnote S. 790).

Miss Adams entdeckte 1924 diesen Sachverhalt aufs neue und präzisierte ihn für Urodelen folgendermaßen: Die Schmelzorgane des Dentale, Praemaxillare, Maxillare und des Praevomer entstammen dem ektodermalen, die des Spleniale und Palatinum dem entodermalen Bereich des Mundepithels. De Beer bestätigte diesen Befund 1947 durch eine peinlich genaue Untersuchung der Ektoderm-Entoderm-Grenze im Mundepithel in vollem Umfang, und unsere Beobachtungen ergeben genau das gleiche Resultat. Wer noch daran zweifelt, betrachte die Querschnitte einer normalen Molchlarve des Gl. Stad. 34–35 und vergleiche die dotterfreien Schmelzorgane im Bereich des Dentale oder Praemaxillare mit den dotterbeladenen im Vomeropalatinum oder Spleniale. Er kann die Richtigkeit der angeführten Tatsache oft in einem und demselben Schnitt bestätigt finden. Es müssen aber unbedingt jüngste Zahnanlagen betrachtet werden, da der Dotter in den Zellen des Schmelzorgans viel schneller aufgebraucht wird als in den übrigen entodermalen Epithelzellen, was bei der intensiven Zellvermehrung im Schmelzorgan leicht verständlich scheint. In den Gl. Stadien 36–37 ist der Unterschied zwischen ektodermalen und entodermalen Schmelzorganen schon viel weniger deutlich. (Zur Unterscheidung von Ektoderm und Entoderm vgl. S. 178!)

Was die *harte Außenschicht* der Zähne, den sog. *Schmelz*, anbetrifft, so hat die alte Auffassung seiner regelmäßigen Abstammung von den Ameloblasten des Schmelzorgans ebenfalls wesentliche Berichtigungen erfahren. Vor allem die zahlreichen genauen Untersuchungen von Schmidt (1938 ff) haben gezeigt, daß die harte Außenschicht an der Zahnspitze durchaus nicht bei allen Wirbeltieren vom Schmelzorgan abstammt. Bei den Säugetieren ist dies zwar sicher, offenbar ohne Ausnahme, der Fall. Bei den Zähnen aller Fische dagegen entsteht die harte Außenschicht nach Schmidt durch Umwandlung von Dentin. Für Amphibien und Reptilien scheint der Sachverhalt noch wenig geklärt zu sein. Kvam (1946) hält den 'Schmelz' der Amphibien (*Triton cristatus* und *Rana*) für mesodermal, d. h. der Zahnpapille entstammend, während er bei Reptilien teils eine mesodermale (*Anguis*), teils eine ektodermale Hartsubstanz (*Lacerta*, *Tarentola*) feststellt. Erler (1935) fand ferner bei Krokodilien, Schmidt (1950) bei einem Stegocephalen echten, d. h. vom Schmelzorgan abstammenden Schmelz.

Kvam (1946) spricht von 'ektodermalem' und 'mesodermalem Schmelz', verwendet also den Begriff 'Schmelz' für jegliche harte Außenschicht an Zähnen ohne Rücksicht auf ihre Genese. Ähnlich wird von Weidenreich (1926) der Unterschied zwischen Schmelz, Dentin und Knochen verwischt. Peyer (1936)

und Schmidt (1949) halten dagegen an einer strikten Unterscheidung von Dentin und Schmelz fest, wobei die Histogenese als Hauptkriterium gilt: der Begriff *Schmelz* wird reserviert für die vom Schmelzorgan gebildete Hartsubstanz, während die aus Dentin durch nachträgliche Auflösung der kollagenen Fasern und stärkere Verkalkung hervorgehenden harten Außenschichten als *Durodentin* bezeichnet werden. Nebst der Histogenese können auch gewisse Merkmale am fertigen Zahn zur Unterscheidung von Schmelz und Durodentin herangezogen werden: echter Schmelz ist viel stärker doppelbrechend als Durodentin und sitzt dem Zahn wie eine Kappe auf, mit einer scharfen Grenze gegen das Dentin und einer Knickung der Zahnkontur am untern Rand dieser Kappe. Demgegenüber geht eine Durodentinspitze kontinuierlich und ohne Knickung der Kontur ins Dentin über.

Die diesbezüglichen Verhältnisse bei unsern Versuchstieren werden im nächsten Kapitel besprochen.

B. *Unterschiede der Zahnbildung bei Triton und Bombinator*

1. *Triton alpestris.* Die Triton-Larven bilden schon vom Gl. Stad. 34 an Zahnanlagen und Zähne in den *Zahnfeldern* des *Praemaxillare* und *Vomeropalatinum* im Oberkiefer, des *Dentale* und *Spleniale* (Operculare) im Unterkiefer (Abb. 2). Vomer- und Palatinum werden zuerst als selbständige Zahnfelder mit

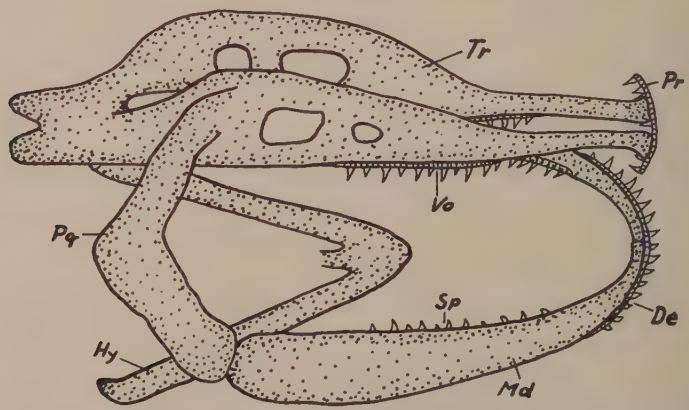

Abb. 2. Kopfskelett und Zahnfelder einer normalen Triton-Larve. Knorpel: *Tr*, Trabecula; *Pq*, Palatoquadrat; *Md*, Mandibulare; *Hy*, Hyoid. Zahnknochen mit Zähnen: *Pr*, Praemaxillare; *Vo*, Vomeropalatinum; *De*, Dentale; *Sp*, Spleniale.

getrennten Verknöcherungen unter der Trabekula angelegt, verschmelzen aber später zu einem einheitlichen bezahnten Knochen, dem Vomeropalatinum. Lateral vom Praemaxillare, vor dem nach außen gerichteten Trabekelhorn, entsteht etwa im Gl. Stad. 42 das kurze *Maxillare*, welches ebenfalls einige Zähne trägt.

Das Mundepithel verdickt sich in den genannten Zahnfeldern zu Zahnleisten, in denen sich die glockenförmigen Schmelzorgane heraussondern. Der Hohlraum der Schmelzorgane wird sofort ausgefüllt von einer Anzahl dicht gepackter plasmaarmer Mesektodermzellen, den *Odontoblasten*, welche die Zahnpapille bilden. Ihre Zahl beträgt zu Beginn nur 4–5, und ihre Anordnung ist durchaus charakteristisch: Zellen und Kerne flachen sich ab und türmen sich mit etwas aufgewölbten Rändern übereinander, ähnlich einem Satz von Suppentellern (Tafel 1c). Der Kern an der Spitze dagegen deformiert sich mit seiner Plasmahülle zu einer langen Spitze, die dem Schmelzorgan von innen dicht anliegt. Im Querschnitt durch die Zahnanlage wird also nur je 1 Odontoblastenkern getroffen. Höchstens an der Basis liegen oft 2–3 wenig oder nicht deformierte Kerne nebeneinander.

Zwischen Odontoblasten und Ameloblasten beginnt nun sofort die Bildung von Hartsubstanz, und zwar entsteht zuerst das sog. Zahnscherbchen aus Praedentin. Es wird in der Azanfärbung lebhaft blau. Im Schmelzorgan tritt erst unmittelbar vor dem Durchbruch der Zähne (erstmals Gl. Stad. 44) eine auffallende Veränderung ein, die auch von Kvam (1946) für *Triton cristatus* beschrieben wird (S. 62 und Abb. 94): Die Ameloblasten vor der Spitze des Zahnscherbchens quellen kräftig auf, so daß zwischen ihren Kernen und der Dentinspitze eine breite, helle Zone entsteht (Tafel 1c). Oft sind in dieser Zone Granula und Schlieren erkennbar, die sich regelmäßig von den Ameloblastenkernen zur Dentinspitze hinziehen. Man gewinnt den Eindruck, daß hier 'etwas' ausgeschieden wird, daß auf jeden Fall die Ameloblasten bei den Veränderungen, die nun die Zahnspitze erfährt, eine Rolle spielen. Ob diese Rolle nun in der Bildung von echtem Schmelz besteht, oder ob nur eine Veränderung in der bereits vorhandenen Dentinspitze bewirkt wird, ist schwer zu entscheiden, da offenbar sowohl bei Zähnen mit echtem Schmelz als auch bei solchen mit Durodentin derartige Bilder vorkommen (vgl. Abb. 57–59 und 117–19 bei Kvam, 1946). Jedenfalls entsteht nun in kurzer Zeit ein kleines Spitzchen aus einer stark doppelbrechenden Hartsubstanz, die ihre gelbe Eigenfarbe auch nach der Azanbehandlung beibehält (Tafel 3b). Kurz darauf bricht der Zahn durch. Das Spitzchen sitzt dem fertigen Zahn als Kappe auf, mit einer deutlichen Störung der Kontur beim Übergang ins Dentin (Tafel 1a). Es bricht denn auch sehr leicht ab. Ein Wachstum nach dem Durchbruch konnten wir nicht feststellen.

Dies alles spricht dafür, daß die kleine Spitzenkappe des Triton-Zahns aus *echtem Schmelz* besteht.

Die fertigen Zähne der Larven sind einspitzig, nach der Metamorphose dagegen werden zweispitzige Zähne gebildet.

2. *Bombinator pachypus.* Die Bombinator-Larven besitzen im Gegensatz zu den Triton-Larven keine Zähne, sondern nur ein kompliziertes System von Hornstiftchen, die mit echten Zähnen morphologisch nichts zu tun haben. Erst während der Metamorphose, also im Alter von mindestens zwei Monaten, wenn das Tier von pflanzlicher zu tierischer Nahrung übergeht, werden echte Zähne

angelegt, aber, wie bei fast allen Anuren,[1] nur im Oberkiefer: im *Praemaxillare*, *Maxillare* und *Vomer*.

Zahnanlagen und Zähne sind bei Bombinator in mehrfacher Beziehung anders gestaltet als bei Triton (Tafel 1 B und D):

Die Papillen der jungen Zahnanlagen sind breit kegelförmig und bestehen aus 12–15 Odontoblasten, die zu mehreren nebeneinander liegen; nur an der äußersten Spitze wird ihre Breite auf 1–2 Kerne reduziert. Im Querschnitt der Zahnanlage werden also fast immer mehrere Kerne zugleich getroffen.

Schon die erste Zahngeneration wird zweispitzig angelegt. In jede Spitze schmiegt sich je 1 Odontoblastenkern hinein.

Das Schmelzorgan liegt dem Dentin dicht auf. Eine Veränderung an seiner Spitze während der Zahnbildung ist nicht zu beobachten, ebensowenig die Anlage einer schmelzartigen Spitze.

Der adulte Bombinatorzahn weist in krassem Gegensatz zum Tritonzahn an seinen 2 Spitzen nur einen sehr dünnen Überzug einer andersartigen Außensubstanz auf, die sich aber vom Dentin nicht scharf abgrenzt wie bei Triton, sondern auch in der Kontur völlig gleichmäßig ins Dentin des Zahnhalses übergeht. Es handelt sich hier offenkundig um *Durodentin* und nicht um echten Schmelz. Das Bild gleicht, abgesehen von der Zweispitzigkeit, den von Schmidt (1951) abgebildeten Fischzähnen mit Durodentin.

Der auffällige Gegensatz zwischen der Spitze des Bombinator- und des Tritonzahnes bestärkt uns in der Auffassung, daß es sich bei Triton wirklich um eine Kappe echten Schmelzes, bei Bombinator aber um eine Schicht Durodentin im Sinne von Peyer (1936) und Schmidt (1949) handelt.

IV. DIE CHIMAERISCHEN ZAHNANLAGEN UND IHRE BEZIEHUNGEN ZU DEN VERSCHIEDENEN ZAHNFELDERN

Vor der Besprechung der neuen Untersuchungsergebnisse seien einige Punkte aus der Arbeit von 1949 rekapituliert.

Das implantierte Neuralleistenmaterial von Bombinator liefert in den Triton-Wirten gute knorpelige Visceralbogen, die sich in Größe, Form und Stellung weitgehend herkunftsgemäß verhalten. So kommen auch solche Knorpelelemente zur Ausbildung, die für Anuren typisch sind, den Urodelen aber fehlen: die Supra- und Infrarostralknorpel, der Processus muscularis Palatoquadrati und die Commissura quadrato-cranialis anterior zwischen Palatoquadrat und Trabecula. Dagegen ist das Bombinator-Mesektoderm nicht imstande, die ebenfalls von der Neuralleiste abstammenden knöchernen Elemente zu ersetzen (vgl. S. 162), die beim Triton-Wirt schon in frühen Larvenstadien auftreten und Träger von Zahnfeldern sind: das Dentale und Spleniale im Unterkiefer, das Praemaxillare und Vomeropalatinum im Oberkiefer. Umso bemerkenswerter

[1] Bezahnte Unterkiefer kommen nur vor bei *Hemiphractidae*, *Amphignathodon*, und *Ceratobatrachus*. Völlig zahnlos sind die Bufoniden sowie die Gattungen *Pipa*, *Dendrobates*, und *Hymenochirus*.

ist die Tatsache, daß das Bombinator-Mesektoderm im Bereich der fehlenden Zahnknochen des Wirtes dennoch echte Zahnanlagen induziert und mit Odontoblasten versorgt.

A. *Entwicklung und Degeneration der einzelnen chimaerischen Zahnanlage*

Die ersten chimaerischen Zahnanlagen erscheinen in den operierten Triton-Embryonen zugleich mit den ersten normalen, im Zahnfeld des Dentale-Infrarostrale (wenn wir auf einen einzelnen Fall abstellen dürfen) sogar etwas vor diesen: in der jüngsten untersuchten Chimaere (Gl. Stad. 34), die auf der unoperierten Seite 6 eben erkennbare normale Zahnanlagen besitzt (davon 2 im Zahnfeld des Dentale), fanden wir auf der operierten Seite schon 7 deutlich erkennbare chimaerische Zahnanlagen (davon 5 im Zahnfeld des Infrarostrale).

Form und *Größe* der chimaerischen Zahnpapillen sowie die Zahl der beteiligten Odontoblasten weicht von der für Triton typischen Norm in gesetzmäßiger Weise ab. Die Breite der Papillen beträgt an der Basis 18–23μ, ist also wesentlich größer als bei den normalen Triton-Papillen (13μ) und stimmt mit den normalen Papillen des metamorphosierenden Bombinators gut überein. Dasselbe gilt angenähert für die *Zahl* der Odontoblasten: während sie in den jungen Triton-Zahnpapillen regelmäßig nur 4–5 beträgt, finden wir in den chimaerischen Zahnpapillen deren 8–12. Auch ihre *Anordnung* ist spendergemäß, indem sie nicht in Einerreihe, sondern auch nebeneinander stehen. Die schmale Spitze des Zahnscherbchens dagegen gleicht dem der normalen Triton-Zahnanlagen, nicht der rundlichen oder schon zweispitzigen Kuppe des (metamorphosierenden) Bombinator-Spenders.

Aus diesen Feststellungen geht deutlich hervor, daß die Odontoblasten nicht nur passives Baumaterial darstellen, sondern daß ihnen — genau wie dem knorpelbildenden Mesektoderm — *eigene aktive Gestaltungskräfte* innewohnen, die bei der Bildung der Zahnanlage wesentlich mitwirken: die Anordnung der Odontoblasten wird von diesen selbst bestimmt. Bei ihrer gegenüber den normalen Spenderpapillen etwas reduzierten Zahl ist wohl das an der Spitze schmalere Schmelzorgan des Triton-Wirtes mit im Spiel.

Die bei der Bildung sofort erreichte Gestalt und Größe der chimaerischen Zahnanlagen wird nun längere Zeit beibehalten. Während bei den normalen Triton-Zahnanlagen das Zahnscherbchen kräftig in die Länge und in die Dicke wächst, nimmt es bei den chimaerischen Zahnanlagen kaum mehr zu und wird nicht dicker als *ca.* 1μ. Am schönsten präsentieren sich die chimaerischen Zahnanlagen etwa 14 Tage nach der Operation im Gl. Stad. 40–41 (Tafel 2B). Während um diese Zeit die ersten Wirtszähne durchbrechen, bleiben die chimaerischen Anlagen im Schmelzorgan stecken, und etwa 19 Tage nach der Operation fangen sie an zu degenerieren.

Die beginnende *Degeneration* zeigt sich zuerst durch eine Auflockerung der vorher dicht gepackten Zellen der Zahnpapille an ihrer Basis (vgl. Tafel 3A). In diesem Stadium fanden wir aber noch keine Pyknosen: die Odontoblasten

lockern sich vor ihrem endgültigen Absterben in Mesenchymzellen auf. (In der Normalentwicklung hätten sie wohl von Anfang an Mesenchym gebildet.) Das Dentinscherbchen wird zuerst an der Basis aufgelöst und dabei öfters durch die sich lockernden Odontoblasten auseinandergedrückt. Das Schmelzorgan flacht sich ab (dies kann auch schon vor der Auflockerung der Papille geschehen) und bleibt zuletzt noch als unregelmäßige Epithelverdickung erkennbar, in der das am längsten persistierende Dentinspitzchen steckt. Auch dieses wird zuletzt vollständig aufgelöst: in den über 30 Tage alten Chimaeren konnten wir keine Spur von chimaerischen Zahnanlagen mehr finden.

Vereinzelt kommen beträchtlich größere chimaerische Zahnpapillen vor, die eine Breite von *ca.* 34μ und eine Höhe von *ca.* 55μ erreichen und *ca.* 25 Odontoblasten enthalten. Bei so auffallend grossen Papillen—wir zahlten im ganzen nur 7 von diesem Typ — handelt es sich immer um solche, die dem Bombinator-Knorpel direkt aufsitzen, während die übrigen durch lockeres Mesenchym von diesem getrennt sind. Auch unter den normalen Triton-Zahnanlagen gibt es übrigens einzelne solche Riesen, und auch sie sind von Anfang an mit dem Knorpel eng verbunden, während die meisten erst sekundär durch Verknöcherungen mit ihm verwachsen.

Diese besonders großen, dem Knorpel aufsitzenden chimaerischen Zahnpapillen verhalten sich auch bei der Degeneration anders als oben beschrieben wurde: ihre Odontoblasten weichen nicht auseinander, sondern zeigen schon früh eine Neigung zu intrapapillärer Kollagenbildung. Ihre Basis verknorpelt

TABELLE 1

Vergleich der chimaerischen mit den normalen Zahnanlagen

	Triton (larval)	*Chimaeren (larval)*	*Bombinator (in Metamorphose)*
Form der Papille	schmal kegelförmig, Odontoblasten in Einerreihe	breit kegelförmig, 2–3 Odontoblasten nebeneinander	breit kegelförmig, mehrere Odontoblasten nebeneinander
Zahl der Odontoblasten	4–5	8–12	12–15
Breite der Papillenbasis	13μ	18–23μ	20μ
Spitze des Zahnscherbchens	*eine* schmale Spitze	*eine* schmale Spitze	zuerst rundliche, dann *zwei*spitzige Kappe
Ameloblasten	vor der Dentinspitze mit granulierten Plasmahöfen	vor der Dentinspitze mit granulierten Plasmahöfen	Kerne dem Dentin dicht anliegend
Spitze des adulten Zahns	vom Dentin scharf abgesetzte, *zwei*spitzige Kappe: *echter Schmelz*	(Zahnanlage bricht nicht durch. Nur Zahnscherbchen aus Prädentin, keine Schmelzkappe).	dünner farbloser Überzug, vom Dentin nicht scharf abgesetzt: *Durodentin*

schließlich und persistiert noch längere Zeit als Warze auf dem Bombinatorknorpel.

B. *Die Verteilung der chimaerischen Zahnanlagen auf die verschiedenen Zahnfelder des Triton-Wirtes*

In unserer Arbeit von 1949 erhielten wir bei 5 operierten Triton-Wirten mit Bombinator-Mesektoderm insgesamt 29 chimaerische Zahnanlagen, 22 im Bereich des praemaxillaren, 7 im Bereich des dentalen Zahnfeldes. Das gegenwärtige Versuchsmaterial lieferte uns bei 13 ausgewerteten Chimaeren insgesamt 91 chimaerische Zahnanlagen, die sich folgendermaßen verteilen:

	Dentale	*Spleniale*	*Praemaxillare*	*Vomeropalatinum*	*Total*
1953 . .	46	18	11	16	91
1949 . .	22	0	7	0	29

Das Mundepithel des Triton-Wirtes ist im Gebiet der chimaerischen Zahnanlagen auch außerhalb der eigentlichen Schmelzorgane stark verdickt, wie es auch in normalen Zahnfeldern der Fall ist. Oft enthalten die chimaerischen Zahnfelder neben gemischten auch noch normale Zahnanlagen mit wirtseigenen Mesektodermpapillen, falls in den entsprechenden Bereichen noch eigenes Mesektoderm vorhanden ist. Aber die Summe der eigenen und der chimaerischen Zahnanlagen bleibt fast immer hinter der in einem normalen Tier zu erwartenden Zahl zurück (vgl. Abb. 3–6). Fehlt eigenes Mesektoderm in einem Zahnfeld vollständig, so wird auch die Bildung normaler Zahnanlagen völlig unterdrückt. Bei drei einseitig operierten Larven der Ausbeute von 1953 wurde auf der operierten Seite kein einziger normaler Zahn angelegt, bei zwei weiteren nur einige wenige im Oberkiefer, nicht aber im Unterkiefer. Eine Chimaere endlich, bei der die beidseitigen Kopfneuralleisten samt der dazwischen liegenden Neuralplatte durch entsprechendes Bombinator-Material ersetzt wurde, bildete im ganzen Kopf keine einzige normale Zahnanlage.

Wir betrachten im folgenden die einzelnen chimaerischen Zahnfelder getrennt. Für jedes überprüfen wir zunächst die Frage, durch welchen Bereich des Bombinator-Mesektoderms es induziert und mit Odontoblasten versorgt wird. Darauf vergleichen wir die chimaerische Zahnbildung mit der normalen auf der unoperierten Kontrollseite des gleichen Tieres oder, für zweiseitig operierte Tiere, bei gleich alten Kontrollarven. Es wird sich zeigen, daß die *Bereitschaft zu chimaerischer Zahnbildung in den einzelnen Zahnfeldern recht veschieden groß ist.*

1. *Zahnfeld des Dentale-Infrarostrale.* An Stelle des fehlenden *Dentale* schnürt sich in den Chimaeren regelmäßig ein Mesektodermstück von der Mandibularspitze mehr oder weniger deutlich ab. Sein Kern bildet einen Knorpel, den man nach Größe, Form und Stellung eindeutig als ein Bombinator-*Infrarostrale* ansprechen kann. Dieser Mesektodermbereich induziert die dem

dentalen Zahnfeld entsprechenden chimaerischen Zahnanlagen und versorgt sie mit Mesektoderm. Die *Stellung* der chimaerischen Zahanlagen ist dieselbe wie die der Zähne am Dentale des Wirtes: ihre Spitzen sind nach vorn und nach oben gerichtet.

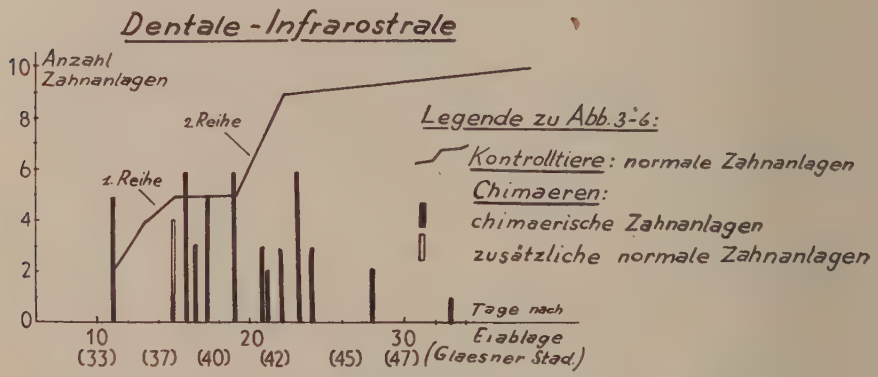

Abb 3. Die Zahnbildung im Zahnfeld des Dentale von 13 operierten Triton-Larven. Jede Säule bedeutet die operierte Seite einer Chimaere. Die ausgezogene Kurve gibt die normale Zahnbildung unoperierter Kontrolltiere an.

Das Zahnfeld des Dentale-Infrarostrale leistet in den Chimaeren weitaus am meisten: die Zahl der chimaerischen Zahnanlagen kommt hier nahe an die Zahl der normalen Zahnanlagen auf der unoperierten Kontrollseite heran, ja bei einigen ganz jung fixierten Keimen liegt sie sogar höher (Abb. 3; 11, 16, 19 Tage). In 10 Fällen, wo die operierte Seite nur chimaerische und keine normalen Zahnanlagen aufweist, stehen insgesamt 42 chimaerische an Stelle von 70 normalen Zahnanlagen. Die über 24 Tage alten Fälle (= über 20 Tage nach der Operation), bei denen schon mit einer Degeneration der chimaerischen Zahnanlagen zu rechnen ist, sind dabei nicht mitgezählt. Die Zahl der chimaerischen Zahnanlagen erreicht also hier 60 prozent des Normalwertes. Betrachten wir nur die Fälle unter 20 Tagen (= 16 Tage nach der Operation), d. h. vor der Bildung der zweiten Zahnreihe im Dentale des Wirtes, die in dem rapiden Anstieg der Kurve in Abb. 3 zwischen 19 und 23 Tagen zum Ausdruck kommt, so finden wir sogar um 14 prozent *mehr* chimaerische Zahnanlagen als normale. Erst in dem Moment, wo der Wirt zur Bildung einer zweiten Reihe von Zahnanlagen übergeht, bleiben die chimaerischen Zahnfelder hinter den normalen zurück. Das Bombinator Mesektoderm macht also die erste Phase der Zahnbildung, nämlich die Bildung der ersten Zahngeneration, vollkommen mit, während es *ca.* 7 Tage später bei der zweiten Phase versagt.

Auffallend ist im Bereich des Infrarostrale auch das fast vollständige Fehlen gemischter Zahnfelder mit chimaerischen *und* normalen Zahnanlagen. In einem einzigen, oben nicht mitgezählten Fall kam eine chimaerische neben drei normalen Zahnanlagen vor. In diesem Fall wurde vom übrig gebliebenen

Triton-Mesektoderm der operierten Seite auch ein knöchernes Dentale gebildet, das in den andern Fällen vollkommen fehlt.

2. *Zahnfeld des Praemaxillare-Suprarostrale.* Ähnlich wie das Dentale wird auch das Praemaxillare in den Chimaeren durch einen den Urodelen fremden, aber für Anuren typischen Knorpel vertreten, nämlich durch ein *Suprarostrale.* Es liegt zwar nicht genau an der Stelle des fehlenden Praemaxillare, sondern *unter* dem Trabekelstab knapp hinter seiner Spitze, gleichgültig, ob diese aus Triton- oder aus Bombinator-Material besteht. Dieser Knorpel bleibt auch in den besten Fällen klein, und erreicht, im Gegensatz zum Infrarostrale, bei weitem nie die für Bombinator typische Größe. Dies erklärt sich aber leicht aus der Tatsache, daß sein Material vom Mandibularbogen her stammt, indem es nämlich von der Unterkieferspitze um das Stomodäum herum sekundär in den Oberkiefer wanderte, also die am weitesten gewanderte Zellgruppe des mandibularen Mesektoderms darstellt (vgl. Wagner, 1949, S. 543–5). Dem Suprarostrale sind die chimärischen Zahnanlagen im Bereich des praemaxillaren Zahnfeldes zugeordnet. Sie sind wie die ersten normalen Zahnanlagen am Praemaxillare nach hinten gerichtet.

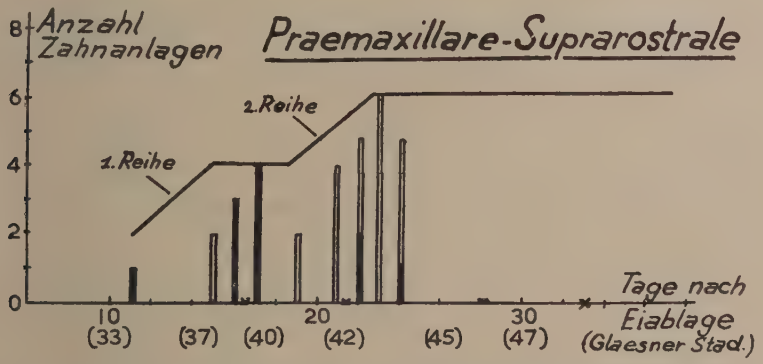

Abb. 4. Die Zahnbildung im Zahnfeld des *Praemaxillare* von 13 Chimaeren. Legende wie Abb. 3. Die Kreuze auf der Nullinie bedeuten Chimaeren ohne jegliche Zahnbildung auf der operierten Seite.

In diesem Zahnfeld fielen bei 6 Chimaeren die normalen Zahnanlagen auf der operierten Seite völlig aus. An ihrer Stelle entstanden insgesamt 8 chimaerische Zahnanlagen, während die unoperierten Kontrollseiten insgesamt 24 normale Zahnanlagen aufweisen (vgl. Abb. 4). Die chimaerische Zahnbildung erreicht also 33 prozent des Normalwertes. Zählen wir nur die 4 Fälle unter 20 Tagen (vor der Bildung der zweiten Zahnreihe des Wirts), so heißen die entsprechenden Zahlen 8 : 14 = 55 prozent. Die Bombinator-Trabekelspitze scheint als Zahninduktor nicht in Frage zu kommen: die chimaerischen Zahnanlagen stehen nie in enger Beziehung zu ihr, sondern ihre Stellung sowie ihre Nachbarschaft zum Suprarostrale geben deutlich ihre Zugehörigkeit zu diesem Knorpel zu

erkennen. Berücksichtigen wir noch die Tatsache, daß das Suprarostrale wegen Materialdefizit auch in den besten Fällen sehr klein bleibt (vgl. oben), so müssen wir dem Mesektoderm des Suprarostrale eine Zahninduktionsfähigkeit zuerkennen, die wohl etwa im gleichen Range steht wie die des Infrarostrale.

3. *Zahnfeld des Spleniale (Operculare).* In der Gegend, wo das Spleniale liegen müßte, induziert der Bombinator-Mandibularstab ein Feld chimaerischer Zähne. Ihre Stellung stimmt auch hier mit der normalen überein: sie sind nach innen und schwach nach oben gerichtet.

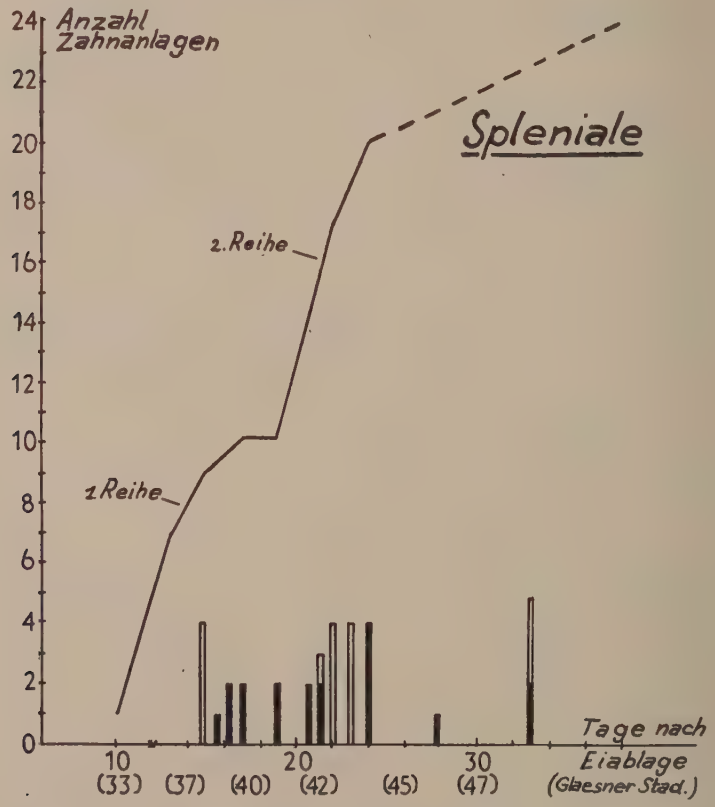

Abb. 5. Die Zahnbildung im Zahnfeld des *Spleniale* von 13 Chimaeren. Legende wie Abb. 3 und 4.

Die chimaerische Zahnbildung bleibt hier aber viel weiter hinter der normalen zurück als im Zahnfeld des Dentale und Praemaxillare (vgl. Abb. 5). Obschon der Triton-Wirt im Feld des Spleniale weit mehr Zähne produziert als im Dentale, kommen doch absolut weniger chimaerische Zahnanlagen vor. 8 Chimaeren mit völligem Ausfall der Wirtszähne brachten insgesamt nur 14 chimaerische an

Stelle von *ca.* 90 normalen Zahnanlagen hervor (= 15 prozent). Auch wenn nur die Fälle unter 20 Tagen berücksichtigt werden (vor der Bildung der zweiten Zahnreihe im Wirt), beträgt der Prozentsatz nur 18 prozent. Auch hier ist keine Zunahme der chimaerischen Zahnbildung in dem Zeitpunkt zu erkennen, wo der Wirt die zweite Zahnreihe anlegt.

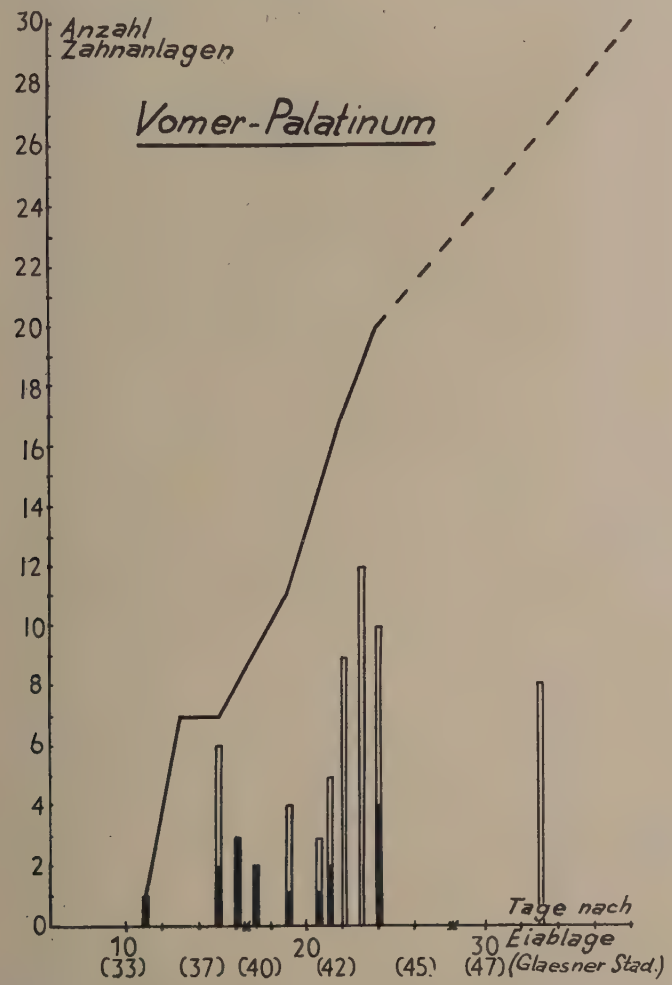

Abb. 6. Die Zahnbildung im Zahnfeld des *Vomeropalatinums* von 13 Chimaeren. Legende wie Abb. 3 und 4.

4. *Zahnfeld des Vomeropalatinums.* Die chimaerischen Zahnanlagen im Bereiche des fehlenden Vomeropalatinums scheinen *nicht* durch das Mesektoderm des Trabekelstabes induziert und mit Odontoblasten versorgt zu werden.

Während nämlich die normalen Zähne des Vomer und Palatinum stets direkt unter dem Trabekelstab liegen und mit der Spitze nach unten (und schwach nach hinten) gerichtet sind, finden sich die chimaerischen Zahnanlagen dieses Bereiches immer auffallend weit vom Trabekelstab entfernt lateral am Mundhöhlendach und sind bald nach vorn, bald nach hinten, bald nach innen gerichtet, aber nie nach unten. Fast immer stehen sie in der Nähe der vom Bombinator-Mandibularbogen aus gebildeten Commissura quadrato-cranialis anterior (vgl. S. 166). Diese erschien in den Chimaeren von 1953 regelmäßig als Fortsatz am Palatoquadrat, der nur in wenigen Fällen die Trabecula erreichte und mit ihr verwuchs. Von hier aus scheinen am benachbarten Rand des potentiellen Zahnfeldes die wenigen chimaerischen Zahnanlagen induziert und mit Mesektoderm versorgt worden zu sein, wodurch auch ihre auffallend unregelmäßige Stellung begreiflich wird.

In 5 Fällen beobachteten wir im Zahnfeld des Vomeropalatinums ein völliges Ausfallen der Wirtszähne. Diese 5 Chimaeren bildeten im fraglichen Gebiet 6 chimaerische an Stelle von *ca.* 40 normalen Zahnanlagen, also nur *ca.* 15 prozent des Verlustes an normalen Zahnanlagen (vgl. Abb. 6). Werden nur die Fälle unter 20 Tagen berücksichtigt, so heißen die Zahlen 5 : 26 = 19 prozent. Wir haben es also hier mit einem schlechten Acker für die chimaerische Zahnbildung zu tun.

5. *Vergleich zwischen den verschiedenen Zahnfeldern.* Im Gegensatz zu unserem Befund von 1949 zeigt es sich, daß nicht allein die Rostralknorpel von Bombinator imstande sind, in Triton chimaerische Zahnanlagen zu induzieren, sondern auch das Mesektoderm des Mandibulare und der Commissura quadrato-cranialis anterior. Die Rostralknorpel geben aber, wie aus der nachfolgenden Gegenüberstellung hervorgeht, auch im gegenwärtigen Versuchsmaterial ihre deutliche Vorrangstellung im Bezug auf Zahninduktionsfähigkeit zu erkennen.

Tabelle 2

Zahnknochen bei Triton	*Induzierender Bombinator-Knorpel in den Chimaeren*	*Zahl der chimaerischen Zahnanlagen verglichen mit der normalen Zahl*	
		Fälle unter 20 Tagen	*alle Fälle*
Dentale	Infrarostrale	114%	60%
Praemaxillare	Suprarostrale	53%	33%
Vomeropalatinum	Commissura quadrato-cranialis anterior	19%	15%
Spleniale	Mandibulare	18%	15%

V. DIE ZAHNANLAGEN AN DEN KIEMENBOGEN DER TRITONLARVEN

Die meisten Urodelenlarven besitzen, wie auch die meisten Fische, an den Kiemenbogen einen sog. *Reusenapparat*, der von den neueren Autoren häufiger als *Siebapparat* oder *Kiemenfilter* bezeichnet wird. Die einzelnen Elemente dieser Einrichtung wurden öfters mit Namen wie 'zahnartige Fortsätze', 'Zähnchen', 'Dentelures', 'Dents cartilagineuses', 'Dentes' bezeichnet (zitiert nach

Stadtmüller, 1924, S. 127), wobei aber mit allen diesen 'Zahn'-Bezeichnungen nur ein Hinweis auf die äußere Form dieser Bildungen, nicht auf ihre wahre Natur gemeint war. Einzig Credner vertrat schon 1886 die Ansicht, daß sie den echten Zähnen entsprechen, 'wie sie auf den Kiemenbogen fossiler Amphibien (Branchiosaurus) wohl bekannt sind' (zitiert nach Stadtmüller, 1924, S. 129). Credner, dessen Feststellung in der späteren anatomischen Literatur offenbar keine Berücksichtigung fand, nahm damit von der rein morphologischen Seite her das Ergebnis vorweg, zu dem Stadtmüller (1924 und 1927) auf Grund eingehender Untersuchungen über die Entwicklung dieser Bildungen gekommen ist:

> Bei der Larve von Salamandra besteht ein Filterfortsatz zunächst aus einer kegelförmigen bindegewebigen Erhebung, die dem Ceratobranchiale aufsitzt. Sie trägt eine Kappe von weicher, hyalin erscheinender Substanz, die kurz als 'Zwischenschicht' bezeichnet werden mag, da sie einerseits an die mesodermale Papille, andererseits an den dritten Bestandteil, die alles überdeckende Epithellage, angrenzt. Ich vergleiche die bindegewebige Erhebung der Zahnpulpa, die 'Zwischenschicht', dem Dentin und den Epithelüberzug dem schmelzbildenden Epithelmantel von Zahnanlagen (Stadtmüller, 1927, S. 384).

Die stoffliche Natur dieser 'Zwischenschicht', die in der Mallory-Färbung genau dasselbe intensive Blau annimmt wie das Dentin der Mundzähne, konnte von Stadtmüller nicht völlig abgeklärt werden. Anorganische Hartsubstanz ließ sich darin nicht nachweisen. Die Vermutung scheint uns nahe zu liegen, daß die Substanz dem ebenfalls noch kalkfreien Praedentin der Mundzähne entspricht (vgl. S. 165). An der Basis der Zahnanlage geht die Zwischenschicht in die Grenzlamelle des Epithels über (Stadtmüller, 1924, und eigene Beobachtung, vgl. Tafel 3C). Eine Schmelzspitze besitzen die Kiemenzähnchen nicht.

Die *Anordnung* der Kiemenzähnchen (wir brauchen fortan diesen, der wahren Natur der Bildungen entsprechenden Ausdruck) ist bei Stegocephalen (Credner, 1886) und bei recenten Urodelenlarven dieselbe (Tafel 3D): der erste Kiemenbogen trägt nur eine 'hinterständige' Reihe nach hinten-innen-oben gerichteter Zähnchen, der vierte nur eine 'vorderständige' Reihe nach vorn-außen-oben gerichteter Zähnchen, während der zweite und dritte Kiemenbogen je eine vorderständige und eine hinterständige Reihe tragen. Die Verhältnisse liegen also in dieser Beziehung ähnlich wie bei den Fischen.

Bei unserem Versuchstier *Triton alpestris* stimmen Bau und Anordnung der larvalen Kiemenzähnchen völlig mit den von Stadtmüller für Salamandra beschriebenen Verhältnissen überein. Die ersten branchialen Zahnanlagen erscheinen bei Triton im Gl. Stad. 38 am ersten und zweiten Kiemenbogen. Ihre Zahl nimmt dann bis zur Metamorphose in der Weise zu, daß zuletzt der zweite und dritte Kiemenbogen am meisten, der erste und vierte am wenigsten Zähnchen besitzen. Eine Zählung anhand der Schnittserien von fünf Larven verschiedenen Alters ergab das folgende Bild (Tabelle 3).

TABELLE 3

Entwicklung der Zahnanlagen an den 4 Kiemenbogen der Triton-Larven

	Links				*Rechts*				
	I	*II*	*III*	*IV*	*IV*	*III*	*II*	*I*	*Summe*
Gl. Stad. 38	1	0+1	0+0	0	0	0+0	0+0	1	3
„ 40	4	0+2	0+0	0	0	0+0	2+0	5	13
„ 44	5	4+5	3+1	1	3	3+4	6+4	5	44
„ 50	7	5+7	5+3	3	3	3+4	4+5	7	56
„ 53	7	6+7	6+6	5	6	6+7	7+6	7	76

In Abb. 7 ist der ausgezählte Fall Gl. Stad. 50 aus der Schnittfolge schematisch rekonstruiert.

Das Hyoid und das ganze Basibranchialskelett tragen keine Zahnanlagen.

Mit der Umwandlung des larvalen zum definitiven Hyobranchialskelett gehen mit den Kiemenspalten auch die Filterfortsätze bei allen Salamandridenformen verloren (Stadtmüller, 1924, S. 132).

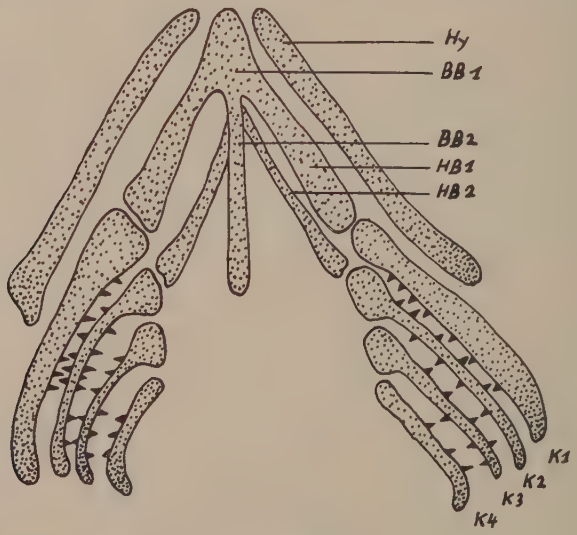

ABB. 7. Die Anordnung der *Reusenzähnchen* an den larvalen Kiemenbogen von Triton alpestris, aufgenommen an einer Larve im Gl. Stad. 50. *K1–K4*, 1.–4. Kiemenbogen (Ceratobranchialia); *HB1*, *HB2*, Hypobranchiale 1 und 2; *BB1*, *BB2*, Basibranchiale (Copula) 1 und 2; *Hy*, Hyoid.

Was nun die *Homologiefrage* dieser Bildungen anbetrifft, so gelangten wir zuerst ganz unabhängig von Credner und Stadtmüller zu der Überzeugung, daß es sich um *echte Zahnanlagen* handelt. Der von Stadtmüller, 1924 und 1927, gegebenen ausführlichen Begründung dieser Auffassung sowie den daraus gezogenen theoretischen Folgerungen können wir in vollem Umfang zustimmen:

Daß gerade bei den Amphibien und hier gerade wieder bei den Urodelen die Filterfortsätze ihre wahre Natur am ehesten erkennen lassen, ist eine schöne Parallele dazu, daß auch das Zahnsystem der Amphibien auf tieferer Stufe als das der Reptilien und Säuger und selbst einer großen Zahl von Knochenfischen steht und daß wir in der Zahnbildung der geschwänzten Amphibien ursprüngliche, ziemlich unverfälschte Verhältnisse vor uns haben, wie O. Hertwig 1874 nachgewiesen hat (Stadtmüller, 1927, S. 384/5).

Es ist vor allem zu denken an das sog. 'plakoide' Stadium in der Entwicklung der ersten Zahngeneration der Teleostier, wie sie Röse 1894 und Burckhardt 1906 schilderten, da wir es ja bei den Filterfortsätzen dieser Salamandridenlarven mit frei hervorragenden Papillen zu tun haben. Die Gebilde sind m. E. auf einer gewissen Stufe der Entwicklung stehen geblieben, zeigen in mancher Beziehung Rückbildungen und sind mit der Übernahme einer andern Funktion, nämlich der eines Filterapparates zum Schutz der Kiemen und im Dienst der Nahrungsaufnahme, in ihrem Bau verändert. Ich erachte sie auch homolog den bei einigen fossilen Amphibien (Stegocephalen) bekannt gewordenen, entsprechend typisch angeordneten Kiemenbogenzähnen, die funktionstüchtige, fertig entwickelte, wirkliche, im Dienst der Nahrungsbewältigung verwendete Hartgebilde darstellen (Stadtmüller, 1927, S. 383).

Unter den Fischen scheint nach Brendecke (1927) der Filterapparat der *Dipnoer* dem der Urodelen am nächsten zu stehen.

Bei den *Knochenfischen* dagegen zeigen die Siebfortsätze einen großen Formenreichtum und im Bau so wechselnde, verwirrende Zustände, daß ihre Natur dort nicht so leicht zu erkennen ist. Sie enthalten dort oft stützende Knocheneinlagen, doch erblickt Zander 1903 in ihnen keine Hartgebilde, die etwa mit den Kieferzähnen verglichen werden könnten (Brendecke, S. 382).

Das Vorkommen echter Zahnanlagen an den Kiemenbogen normaler Triton-Larven bestätigt von der rein morphologischen Seite den durch Transplantationsexperimente gewonnenen Befund Sellman's, daß eine geringe Zahnbildungstendenz auch in de hinteren Visceralbogen vorhanden ist (vgl. S. 180). Die Sonderstellung des Hyoids, die Sellman nicht erwähnt, müßte allerdings noch näher untersucht werden.

Wir werden bei der theoretischen Besprechung der chimaerischen Zahnbildung auf die hier beschriebenen Verhältnisse zurückkommen (vgl. S. 179 f.).

VI. SINNESKNOSPEN

Längs der Branchialbogen kommen in den Triton-Larven Reihen von Sinnesknospen vor. Sie finden sich oft unmittelbar neben den Kiemenzähnchen, unterscheiden sich aber morphologisch immer deutlich von ihnen (Tafel 3D). Längs des Hyoides, das keine Zahnanlagen trägt, läuft eine von lockerem Mesenchym erfüllte, gegen die Mundhöhle zu vorragende Schleimhautfalte. In ihrer Kammlinie ist eine Verdickung der Basalmembran festzustellen, und es treten dort auch Sinnesknospen auf.

Diese Sinnesknospen liegen im *entodermalen* Teil des Mundepithels, passen

also nicht in die Lehre der Keimblattspezifität. Es fehlt aber nicht an Theorien, um ihre ektodermale Abstammung zu retten:

Reisinger (1933) analysierte die Sinnesknospen im Mund von Alytes und Rana und erklärt ihre perzipierenden Elemente für den entodermalen Teil des Mundepithels als Abkömmlinge des Mesektoderms, während sie im ektodermalen Mundepithel ganz aus diesem hervorringen. Mesektodermzellen der Visceralbogen wandern nach Reisinger ins entodermale Epithel ein. Reisinger kam zu dieser Behauptung auf Grund von histologischen Beobachtungen und von Defektversuchen: Larven mit exstirpierter Neuralleiste, denen das Visceralskelett fehlte, besaßen auch keine Sinnesknospen. Er schloß daraus auf eine materielle Beteiligung des Mesektoderms an der Bildung der Sinnesknospen. Der experimentelle Nachweis dieser Behauptung durch Transplantationsversuche gelang ihm allerdings nicht.

Marcus (1930) führt sogar die gesamte 'viscerale Sinnesschicht' auf eingewachsenes Ektoderm zurück und erklärt den hohen Dottergehalt ihrer Zellen dadurch, daß diese 'vermutlich Dotterkörner vom Entoderm aufnehmen' (l. c., S. 430) und dadurch einen 'entodermalen Habitus' (S. 432) erhalten. Der Dottergehalt wäre demzufolge kein brauchbares Kriterium zur Unterscheidung von Ektoderm und Entoderm. Aber auch diese Hypothese von Marcus wird weder durch Experimente noch durch genaue Beobachtungen gestützt. Sie wird durch den Befund von Holtfreter (1933), der im Entoderm total exogastrulierter Ambystoma-Keime eine schöne 'viscerale Sinnesschicht' mit Sinnesknospen erhielt, klar widerlegt.

Übrigens haben schon Kerr (1903), Landacre (1907), Keibel (1913), Greil (1914) u. a. eine entodermale Entstehung von Sinnesknospen (und zum Teil von Schmelzorganen) angenommen.

Unsere eigenen xenoplastischen Neuralleisten-Chimaeren, besonders das Versuchsmaterial von 1949, wo wir auch die Neuralleiste der Kiemenregion transplantierten, sind dazu geeignet, die These von Reisinger zu überprüfen. Ist sie richtig, so müssen in den Triton-Chimaeren mit reinem Bombinator-Visceralskelett entweder chimaerische Sinnesknospen auftreten, oder die Sinnesknospen müssen gänzlich ausfallen. Statt dessen treten aber schöne Reihen normaler Triton-Sinnesknospen auf. Eine Einwanderung von Bombinator-Zellen in das Triton-Epithel müßte leicht erkennbar sein, fehlt aber vollkommen. Wohl gibt es in normalen und chimaerischen Embryonen Zonen innigsten Kontaktes zwischen Mesektoderm und Epithel (vgl. Wagner, 1949, Abb. 24–31), die eine Mesektodermeinwanderung ins Epithel vortäuschen könnten. Aber die genannten Befunde an den Chimaeren sprechen eindeutig gegen Reisinger's Auffassung. Seine Beobachtung, daß die Sinnesknospen bei skelettlosen Larven fehlen, beweisen wohl die *induktive* Notwendigkeit des Neuralleistenmaterials, aber keineswegs seine materielle Beteiligung an den Sinnesknospen. Andrerseits zeigen unsere Chimaeren, daß das Bombinator-Mesektoderm ebenfalls imstande ist, im Triton-Mundepithel Sinnesknospen zu induzieren.

Die Sinnesknospen verhalten sich also bezüglich ihrer Herkunft genau wie die Schmelzorgane: sie sind teils ektodermaler, teils rein entodermaler Herkunft. Schmelzorgane und Sinnesknospen stellen zwei verschiedene Variationen des vielseitigen Themas 'Epithelreaktion auf Mesektodermkontakt' dar.

Die Existenz rein entodermaler Sinnesknospen muß als weitere Einschränkung der Lehre von der Keimblattspezifität endgültig hingenommen werden (vgl. S. 162–3).

VII. THEORETISCHES ZUR ZAHNBILDUNG

A. *Die Faktorenkette der normalen Zahnbildung*

Auf Grund der experimentellen Befunde früherer Autoren (Holtfreter, 1935; Woerderman, 1946; Sellman, 1946; Wagner, 1949, u.a.) ergibt sich für die Zahnbildung etwa folgende mutmaßliche Faktorenkette:

> Die primäre Induktion geht vom Entoderm des Vorderdarms aus und gibt einzelnen Mesektodermbereichen Zahnbildungstendenz. Von diesen aus wird die Epidermis zur Bildung von Schmelzorganen induziert. Diese wirken ihrerseits als lokale Zentren zurück auf das Mesektoderm und induzieren die Anhäufung von Dentinbildnern in Zahnpapillen (Baltzer, 1950, S. 464).

Die einzelnen Glieder dieser Faktorenkette lassen sich durch folgende experimentelle Ergebnisse belegen:

(*a*) *Notwendigkeit des oralen Entoderms*: Adams (1931) konstatierte nach Exstirpation von oralem Entoderm Ausfälle in der Zahnbildung sowohl in den Zahnfeldern mit entodermalen wie in denen mit ektodermalen Schmelzorganen, obschon bei den letzteren das Anlagematerial selbst nicht angetastet war. Sellman (1946) erhielt bei *Ambystoma mexicanum* durch homoplastische Transplantation von oraler Epidermis und oralem Mesektoderm in den Rumpf Zahnanlagen, wenn er auch orales Entoderm mittransplantierte, andernfalls nicht (l. c., S. 102 und 121).

(*b*) *Notwendigkeit des Mesektoderms* für die Bildung von Schmelzorganen: bei Defekten im Mesektoderm (Exstirpation von Kopfneuralleiste) geht die Zahl der Zahnanlagen zurück, ohne Mesektoderm fallen ganze Zahnfelder vollständig aus. Und zwar bleibt das Epithel dann völlig inaktiv und bildet weder Verdickungen noch Schmelzorgane (Stone, 1926; Raven, 1931; Hörstadius & Sellman, 1945, S. 158 f.; Sellman, 1946, S. 105; Andres, 1946; Wagner, 1949, S. 552 und das gegenwärtige Versuchsmaterial).

(*c*) *Notwendigkeit des Epithels* für die Bildung von Zahnpapillen: nach Exstirpation von Mundektoderm fallen die Zähne im Bereich des ektodermalen Mundepithels (Dentale, Praemaxillare, Vomer) weitgehend aus, während die Zähne im entodermalen Mundepithel (Spleniale, Palatinum) in normaler Zahl erscheinen (Adams, 1924; Sellman, 1946, S. 111). Offenbar bildet das Mesektoderm ohne reagierendes Epithel auch keine Papillen aus. In unseren eigenen Bombinator-Chimaeren von 1949 zeigt

implantiertes Triton-Mesektoderm keine sicheren Ansätze einer Zahnpapillenbildung, da ja die Bombinator-Epidermis 'nicht mitmacht' (Wagner, 1949, S. 553).

Die Punkte (*b*) und (*c*) dieser Faktorenkette können als gesichert betrachtet werden. Woerdeman, 1946, spricht sehr treffend von einem 'reciprocal inductive influence between the epithelial enamel-organ and mesenchymal dental papilla' (l.c., S. 14-15). Dagegen erweist sich Punkt (*a*) bei genauerer Überprüfung als zu wenig abgeklärt. Miss Adams (1931) selbst ist auf Grund ihrer Experimente von einer induktiven Wirksamkeit des Entoderms nicht überzeugt:

> . . . the decrease in numbers of ectodermal tooth germs, although none of this layer was removed, is perhaps due to improper arrangement of the potential tissues rather than to lack of potency (l.c., S. 161).

Die Drehungsversuche von Hörstadius & Sellman (1946) und Sellman (1946) beweisen zudem, daß schon in den einzelnen Zonen der Neuralleiste Unterschiede sowohl in der Knorpel — wie in der Zahnbildungspotenz festgelegt sind ('perhaps . . . influence from the underlying tissue', Sellman, S. 108): die Zahnbildungstendenz ist am größten im präsumptiven Mandibular- und Trabekularbogen, weit kleiner, aber doch noch vorhanden in den übrigen präsumptiven Visceralbogen (vgl. Kiemenzähnchen, S. 174–7).

In den Experimenten von Balinsky (1947) wurden bei völligem Fehlen der entodermalen Kiementaschen auch keine knorpeligen Kiemenbogen gebildet. Und zwar wird der Knorpel

> . . . Stück für Stück unter dem Einfluß des benachbarten entodermalen Epithels gebildet. . . . Man bekommt den Eindruck, daß das Epithel der Kiementaschen die Fähigkeit hat, Zellen des Mesektoderms an seiner inneren Oberfläche zu sammeln und sich verknorpeln zu lassen (l.c., S. 378).

Der Kieferbogen aber verhielt sich (wie auch Mangold, 1936 und 1950 bemerkt) anders: das Quadratum entwickelt sich nach Balinsky unabhängig vom Entoderm, während die Ausbildung des Mandibulare (Cartilago Meckeli) von der ektodermalen Mundeinstülpung abhängig ist. Nun kann nach Balinsky das präsumptive Mundektoderm unter dem Einfluß 'regionaler Faktoren' auch ohne Entodermkontakt eine zwar unvollkommene Einstülpung bilden, die aber für das Zustandekommen eines Mandibularknorpels genügt. Transplantiertes Mundektoderm dagegen tut dies nur unter dem Einfluß von mittransplantiertem Mundentoderm.

Diese Feststellung erklärt vollkommen den oben genannten Befund von Sellman (1946), der bei Transplantation von Mesektoderm und Mundektoderm in den Rumpf nur dann Zähne erhielt, wenn auch orales Entoderm mittransplantiert wurde: nur in diesem Fall entstand nämlich eine Mundeinstülpung, womit erst die Voraussetzung für die Bildung eines als Mandibulare zu deutenden Knorpels und damit auch für die Zahnbildung geschaffen war.

Damit ist aber das Kopfentoderm in der direkten Faktorenkette der Zahnbildung weitgehend degradiert: es beeinflußt sie nur indirekt durch seine ordnende Wirkung, die ja für die Entstehung der gesamten Kopftopographie anerkanntermaßen von überragender Bedeutung ist.

Ob für die Zähne des Oberkiefers dasselbe gilt wie für die des Unterkiefers, bleibt allerdings dahingestellt: die Arbeit von Balinsky (1947) enthält leider keine Angaben über die Abhängigkeit des Trabecularbogens vom Entoderm.

Fällt nun das Entoderm als Glied in der induktiven Faktorenkette der Zahnbildung aus, so muß diese noch weiter nach rückwärts verlängert werden, besitzen doch, wie oben erwähnt, schon die einzelnen Abschnitte der Neuralleiste verschiedene Zahnbildungstendenzen. Diese können wohl nur durch das unterlagernde Urdarmdach induziert worden sein.

B. *Die chimärische Zahnbildung*

Wie wir in Kapitel IV ausführten, ist das Bombinator-Mesektoderm imstande, im Triton-Wirt *induktiv und materiell* an der Bildung von Zahnanlagen teilzunehmen. Diese Potenzen des Bombinator-Mesektoderms scheinen auf den Mandibularbogen beschränkt zu sein. Denn nicht nur die chimaerischen Zahnanlagen im Unterkiefer, sondern auch diejenigen im Oberkiefer wurden durch Mesektoderm des Mandibularbogens induziert und mit Odontoblasten versorgt. Das Material des Suprarostralknorpels, das die chimaerischen Zahnanlagen an der Stelle des fehlenden Praemaxillare induziert, entstammt nämlich der Spitze des Mandibularbogens (vgl. S. 171), und die chimaerischen Zahnanlagen am Mundhöhlendach, im Bereich des potentiellen Zahnfeldes des Vomeropalatinums, wurden vermutlich ebenfalls durch einen Fortsatz des Mandibularbogens induziert, nämlich durch die Commissura quadrato-cranialis anterior (vgl. S. 173–4).

Demgegenüber gab das Mesektoderm der Bombinator-Trabecula in keinem einzigen sicheren Fall zu chimaerischer Zahnbildung Anlaß.

Beim normalen metamorphosierten Bombinator liegen die Verhältnisse gerade umgekehrt: Zähne besitzt nur der Oberkiefer, der Unterkiefer bleibt zeitlebens unbezahnt. Mandibulares Mesektoderm des Infra- und Suprarostrale induziert dafür im frühen Larvenstadium die 'Mundbewaffnung' der Hornzähnchen. Solche Hornzähnchen vermag aber umgekehrt auch der Triton-Wirt in Anurenepidermis zu induzieren (Spemann & Schotté, in Spemann, 1936; Holtfreter, 1935 *a*, *b*; Henzen noch unveröffentlicht), und vermutlich ist auch dort das mandibulare Mesektoderm der wirksame Faktor. Der frühlarvale Induktor (das Stichwort 'Mundbewaffnung', Spemann, 1936, S. 237) wäre also bei Anuren und Urodelen derselbe, total verschieden ist aber das Verhalten des Reaktors, nämlich des Epithels, auf diesen gleichen Reiz. Oder in der Ausdrucksweise von Baltzer (1950): der Induktor stellt die allgemeine, der Reaktor die spezifische Komponente dar.

Zeitlich ist die Zahninduktionsfähigkeit des Bombinator-Mesektoderms auf

eine ganz kurze Phase während der frühen Larvenentwicklung beschränkt. Wie aus den Abb. 3–6 und dem zugehörigen Text. S. 169–74 hervorgeht, entstehen alle chimaerischen Zähne im Alter von 11–15 Tagen des Triton-Wirtes (7–11 Tage nach der Operation).[1] Während die Zahl der wirtseigenen Zähne später mit der Bildung weiterer Zahnreihen stufenweise zunimmt, ist keine Zunahme der chimaerischen Zahnanlagen mehr zu konstatieren. Die Möglichkeit läßt sich zwar nicht ganz von der Hand weisen, daß es sich dabei bereits um eine Vorstufe der Degeneration des Bombinator-Mesektoderms handelt, obschon diese erst im Alter von etwa 23 Tagen (19 Tage nach der Operation) erkennbar wird. Wir halten aber eher dafür, daß das Bombinator-Mesektoderm die Fähigkeit der Induktion von 'Mundbewaffnung' tatsächlich nur in der genannten kurzen Zeitspanne besitzt, die einem Alter des Bombinator-Spenders von 8–13 Tagen nach der Eiablage entspricht: Genau in diesem Stadium werden bei normalen Kontrolltieren die Hornzähnchen angelegt! In den Triton-Wirten mit Bombinator-Mesektoderm läßt sich übrigens nebst der chimaerischen Zahnbildung eine vermehrte Hornbildung des Mundepithels beobachten (Wagner, 1949, S. 554 f.).

Zusammengefaßt:

	im Triton-Mundepithel	*im Bombinator-Mundepithel*
Bombinator-Mesektoderm induziert	echte Zahnanlagen, vermehrte Hornbildung	Hornstiftchen
Triton-Mesektoderm induziert	echte Zahnanlagen	Hornstiftchen

Die paradoxe Tatsache, daß in den Triton-Chimaeren *nur mandibulares*, nie trabeculares Bombinator-Mesektoderm Zahnpapillen liefert, während im adulten Bombinator nur der Bereich des Trabecularbogens, *nie der des Mandibularbogens* bezahnt ist, läßt zwei Deutungsmöglichkeiten offen.

Einmal läßt sich zwischen dem Mesektoderm des Trabecular- und des Mandibularbogens von Bombinator eine starke zeitliche Verschiebung der gleichartigen Induktionsfähigkeit für 'Mundbewaffnung' annehmen: das mandibulare Mesektoderm besitzt sie nur während einer frühlarvalen Phase, wo das Epithel mit der Bildung von Hornstiftchen reagiert. Das trabekulare Mesektoderm dagegen erlangt diese Induktionsfähigkeit erst zur Zeit der Metamorphose, und jetzt reagiert das Mundepithel mit der Bildung von Schmelzorganen.

Es ist aber auch denkbar, daß das trabekulare Mesektoderm der Anuren überhaupt keine Zahnbildungsfähigkeit besitzt, und daß die Zahnbildung beim metamorphierenden Anur eine phylogenetische Neuerwerbung darstellt, die einem andern Entwicklungsmechanismus folgt als bei den Urodelenlarven. Die Zahnbildung von Anur und Urodel wäre dann nicht mehr homodynam im Sinne von Baltzer (1950). Daß die Zahnpapillen dem Mesektoderm entstammen, ist ja vorläufig nur für larvale Urodelenzähne bewiesen, und es ist kaum statthaft, für die Zähne der metamorphierenden Anuren zum vornherein dasselbe

[1] Alter des Operationsstadiums von Triton 4 Tage, von Bombinator 2 Tage nach der Eiablage.

anzunehmen. Hier werden erst experimentelle Untersuchungen der Vorgänge während der Metamorphose einzusetzen haben.

In diesem Zusammenhang mag auch der auf Seite 165–6 beschriebene Befund bedeutsam sein, daß die Triton-Zähne echten Schmelz, die Bombinator-Zähne aber nur Durodentin besitzen. Entweder müssen wir bei dem Zahn des phylogenetisch jüngeren Anurentyps einen sekundären Verlust des Schmelzes annehmen, oder wir fassen ihn als eine schmelzlose Neuerwerbung auf. Entwicklungsphysiologische Homodynamie spräche für die erste, Nicht-Homodynamie für die zweite Annahme.

So interessant das Zustandekommen chimaerischer Zahnanlagen vom entwicklungsphysiologischen Standpunkt aus erscheint, so eng begrenzt ist doch ihre morphologische Leistungsfähigkeit. Mit der Bildung der typischen, aus Bombinator-Papille und Triton-Schmelzorgan bestehenden Zahnanlage mit einem dünnen, vermutlich aus Praedentin bestehenden Zahnscherbchen ist das Maximum der Entwicklung erreicht: die chimaerische Zahnanlage wird niemals zu einem Zahn.

Ob das Zahnscherbchen bei normalen und chimaerischen Zahnanlagen eine Leistung der Zahnpapille ist, scheint uns zum mindesten fraglich. Die Befunde an den chimaerischen Zahnanlagen legen die Vermutung nahe, daß es vom Schmelzorgan gebildet werden könnte. Einmal hat ihr Zahnscherbchen immer die für Triton, nicht die für Bombinator typische Form, diese wird also durch das Schmelzorgan, nicht durch die Papille, bestimmt (vgl. S. 167). Zum andern gehen die chimaerischen Zahnanlagen über die Bildung eines Zahnscherbchens niemals hinaus, offenbar deshalb, weil die Bombinator-Papille unfähig ist zur Bildung von Dentin, eines Stoffes, der ja der Bombinator-Larve überhaupt fremd ist, erscheinen doch auch die ersten Verknöcherungen bei Bombinator erst während der Metamorphose.

Ähnlich wie die chimaerischen Zahnanlagen verhalten sich in dieser Beziehung die Zähnchen an den Kiemenbogen der Triton-Larven (vgl. S. 174 ff.). Auch dort handelt es sich um Mesektodermbereiche, die kein Knochenmaterial liefern, und auch bei jenen Zahnanlagen reicht es nur zur Bildung einer kalkfreien, wohl dem Praedentin gleichzusetzenden Kollagensubstanz, die an ihrer Basis in die Grenzlamelle übergeht.

Als letzter Schritt folgt beim normalen Tritonzahn die Bildung der Schmelzkappe (vgl. S. 165). Bei den chimaerischen Zahnanlagen entsteht zwar vor der Spitze des Zahnscherbchens im Schmelzorgan meist auch ein weiter, granulierter Plasmahof (vgl. Tafel 2B) wie bei den normalen Triton-Zahnanlagen, aber zur Bildung einer Schmelzkappe kommt es nie. Eine Ursache dafür liegt vielleicht in der mangelnden Dentinbildung der Zahnpapille, die, statt kräftig in die Länge zu wachsen und das Schmelzorgan zu durchbrechen, in der Tiefe stecken bleibt.

Die chimaerischen Zahnanlagen gleichen also in mehrfacher Beziehung den Kiemenzähnchen der Triton-Larven: beide Bildungen bleiben auf dem Anfangs-

stadium der Zahnentwicklung stehen, indem sie *erstens* kein Dentin, sondern nur eine kalkfreie oder äußerst kalkarme Kollagensubstanz (Praedentin ?) produzieren, *zweitens* keine Schmelzkappe erhalten und *drittens* das Schmelzorgan nie durchbrechen. Beide Bildungen verfallen auch nach einer gewissen Zeit der Degeneration.

Die Ursache dieser Ähnlichkeit liegt vermutlich darin, daß in beiden Fällen Mesektoderm beteiligt ist, das zwar noch die Fähigkeit der Zahninduktion und Papillenbildung, aber nicht mehr die der Dentinbildung besitzt.

Ist die Fähigkeit der Zahninduktion schon bei Triton in den Kiemenbogen bedeutend schwächer als in den Kieferbogen, so scheint sie dem Kiemenmesektoderm von Bombinator ganz zu fehlen: in unsern Triton-Chimaeren mit Bombinator-Kiemenbogen (Material von 1949) wurden keine chimaerischen Kiemenzähnchen gebildet.

Es wäre höchst wünschenswert, das Neuralleistenmaterial einer völlig zahnlosen Anurenart (vgl. Fußnote S. 166) durch xenoplastische Transplantationen auf seine Zahninduktionsfähigkeit zu prüfen. Unsre theoretischen Überlegungen legen die Vermutung nahe, dass der Versuch auch dort positiv ausfallen würde.

VIII. SUMMARY

1. *The experiment (pp. 161–2)*

A piece of the neural crest containing the presumptive trabecular, mandibular, and hyoid arches was exchanged between early neurulae of *Triton alpestris* and *Bombinator pachypus*. As a consequence, cartilaginous visceral arches and tooth papillae of Bombinator mesectoderm were formed in the Triton hosts (only these are considered).

2. *Comparison of normal tooth formation in Triton and Bombinator (pp. 162–6)*

(*a*) *The larvae.* Triton larvae possess real teeth, which are attached to true dental bones: in the upper jaw to the premaxillary, maxillary, vomerine, and palatine (the last two forming the vomero-palatine); in the lower jaw to the dentary and the splenial. The enamel organs of these teeth arise, as Adams showed in 1924 and de Beer in 1947, partly from the ectodermal area (dentary, premaxillary, maxillary, vomerine), partly from the endodermal area (splenial, palatine) of the mouth epithelium.

On the other hand, Bombinator larvae do not possess any true teeth up to metamorphosis. Only during metamorphosis are real teeth formed in premaxillary, maxillary, and vomerine. The lower jaw remains entirely without teeth.

(*b*) *The adult teeth.* The problem of enamel and dentine, i.e. the problem of the origin of the hard superficial layer of the teeth (the classical 'enamel'), was settled by the researches of Schmidt (1938 and subsequently) for fishes. This group of animals completely lacks real enamel, i.e. enamel originating in the enamel organ. The hard exterior layer of their teeth consists of transformed dentine and is called 'durodentine'.

Reptiles and mammals certainly possess real enamel. For amphibians the situation is not entirely clear. A critical comparison between the adult teeth of Triton and Bombinator shows that Triton possesses a cap of real enamel, whereas Bombinator only possesses a covering of durodentine.

3. *The mixed tooth germs (pp. 166–74)*

(*a*) *Formation, shape, and degeneration of the individual tooth-germ.* The mixed tooth germs appear as early as the first row of teeth of the Triton host. No more mixed tooth formation takes place during the formation of the second and third row of the host. The mixed tooth germs consist of an ectodermal or endodermal enamel organ of the Triton host and of a papilla of Bombinator mesectoderm. A thin layer of predentine (the 'Zahnscherbchen') ending in a long point is formed between the two tissues. Arrangement and number of the Bombinator odontoblasts are to a great extent donor-like, whereas the shape of the point of the 'Zahnscherbchen' is host-like. The mixed tooth-germs never break through, but begin to degenerate about 19 days after operation: the odontoblasts loosen and become mesenchymal cells before their necrosis, the enamel-organ becomes flat and the 'Zahnscherbchen' is dissolved. Some particularly big tooth-papillae fuse with the Bombinator cartilage and are transformed into cartilage instead of mesenchyme during the degeneration of the tooth-germs.

(*b*) *The dissimilar behaviour of the different tooth areas.* In the operated Triton larvae the dental bones are lacking completely. As is known, they too originate in the mesectoderm (cf. Sellman, 1946, p. 122; Wagner, 1949, pp. 542 f.). Instead, the Bombinator mesectoderm forms, in the Triton hosts, two cartilaginous elements typical of anurans but lacking in the urodeles: an infrarostral cartilage instead of a dentary bone, and a less perfect suprarostral cartilage instead of the premaxillary bone. Mixed tooth germs were formed most regularly near these two cartilaginous areas. Such mixed tooth germs are also formed, but less frequently, in the area of the absent splenial and palatine. Closer analysis shows that all mixed tooth papillae originate in the mesectoderm of the mandibular arch. That is to say that the suprarostral cartilage originates in the most rostral part of the mandibular arch (Wagner, 1949, pp. 543 f.), and that also the tooth germs in the area of the palatine are very probably not formed from the trabecula, but from the commissura quadrato-cranialis anterior, belonging to the mandibular arch.

So it is seen that only the mandibular arch of Bombinator, which in normal development always remains toothless, induces tooth germs in Triton. This paradoxical fact is discussed on p. 182.

4. *The tooth germs on the branchial arches of the Triton larvae (pp. 174–7)*

In agreement with Credner (1886) and Stadtmüller (1924) we are convinced that the 'Filterfortsätze' on the branchial arches of the Triton larvae are real

tooth germs. They are of theoretical interest here, because they remain in a similarly low grade of realization ('Realisationsstufe', Lehmann, 1945) as the mixed tooth germs and, like these, degenerate after a certain time.

5. *Taste buds (pp. 177–9)*

Reisinger (1933) maintains that the taste buds in the endodermal part of the mouth epithelium are formed by immigration of mesectoderm into the epithelium. This opinion is disproved by our chimeras. Taste buds, as well as enamel organs, can be formed by the endodermal epithelium (in confirmation of Landacre, 1907; Holtfreter, 1933; de Beer, 1947).

6. *The inductive factors of tooth formation (pp. 179–84)*

The two formative elements of the tooth germs, mesectoderm and mouth epithelium, influence each other during the formation of the individual tooth germ in a 'reciprocal inductive influence' (Woerdeman, 1946, pp. 14–15). Each tissue remains inactive without the other, but together they achieve a real 'kombinative Einheitsleistung' (Lehmann, 1945). The role of the endoderm seems restricted to its general topographically arranging influence (formation of the branchial fissures and of the mouth opening), without which the normal migration and differentiation of the mesectoderm—and, as a consequence, tooth formation—do not take place (Mangold, 1936, 1950; Balinsky, 1947). A first determinative influence must be ascribed to the archenteron roof, because differences in tooth-forming potency seem to be already present in the neural crest (Sellman, 1946).

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ERKLÄRUNG ZU DEN TAFELN

TAFEL 1

A. Normaler Zahn eines *adulten Triton alpestris* (Praemaxillare). Ungefärbtes Totalpräparat im Phasenkontrastmikroskop.

B. Normaler Zahn eines *adulten Bombinator pachypus* (Vomer). Ungefärbtes Totalpräparat im Phasenkontrastmikroskop.

C. Zwei *normale Zahnanlagen* einer *Triton*-Larve (Gl. Stad. 41) im Zahnfeld des Spleniale. *Am*, Ameloblasten (Schmelzorgan); *S*, beginnende Schmelzbildung; *Z*, Zahnscherbchen; *Od*, Odontoblasten (Zahnpapille); *Md*, Mandibulare.

D. *Normale Zahnanlage* einer metamorphosierenden *Bombinator*-Larve (mit eben durchgebrochenen Vorderbeinen) im Zahnfeld des Praemaxillare. Bezeichnungen wie B.

TAFEL 2

A. Querschnitt durch den Kopf einer Triton-Larve (Gl. Stad. 40) mit implantierter Bombinator-Neuralleiste. Links normale Seite mit Triton-Knorpeln und normalen Zahnanlagen (*ZA T*), rechts operierte Seite mit Bombinator-Knorpeln und *chimärischen Zahnanlagen* (*ZA Bo*). Rechts fehlen normale Zahnanlagen vollständig. *Tr*, Trabecula; *Md*, Mandibular; *IR*, Infrarostrale. (Konturen z. T. verstärkt).

B. Detail aus Bild A: Zwei *chimaerische Zahnanlagen* im Längsschnitt. *Am T*, Triton-Ameloblasten; *Od Bo*, Bombinator-Odontoblasten; *Z*, Zahnscherbchen. Übrige Bezeichnungen wie A.

TAFEL 3

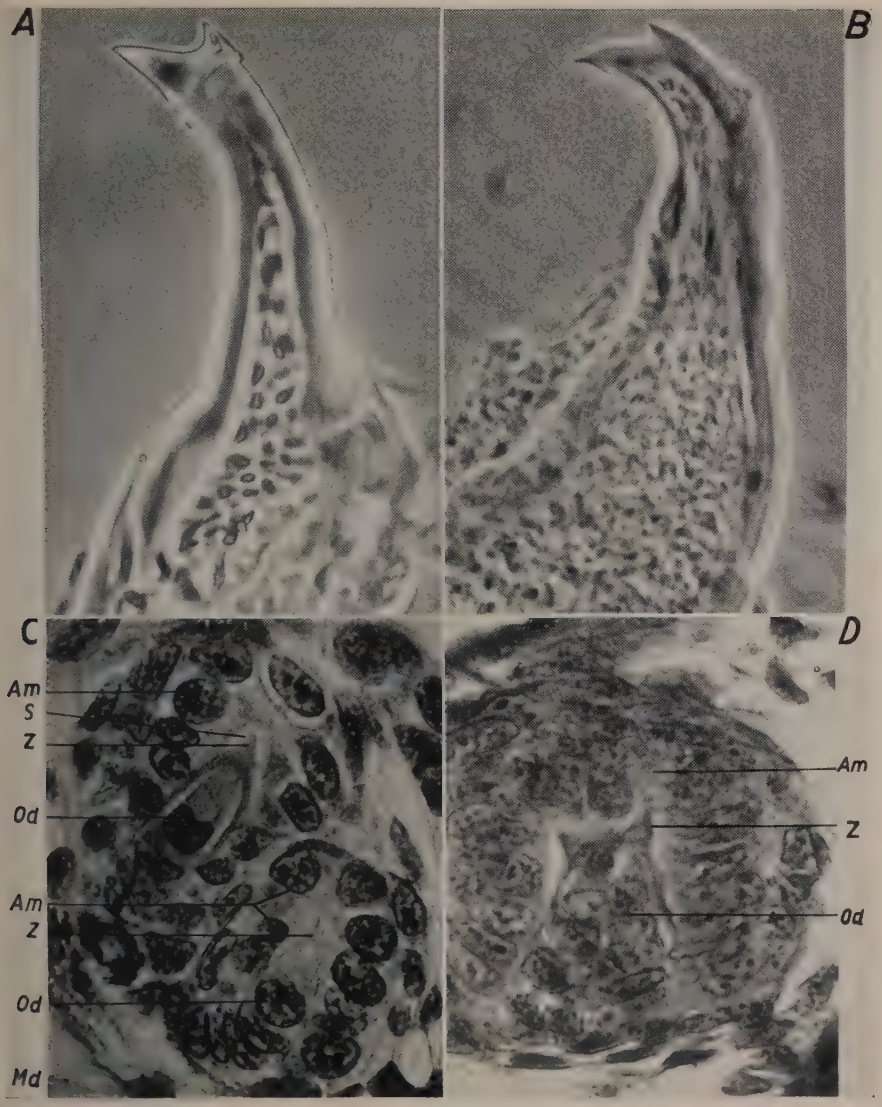
A. *Degenerierende* chimaerische Zahnanlage in einer Triton-Larve Gl. Stad. 43. Bombinator-Papille (*Od Bo*) in Auflockerung; Triton-Schmelzorgan (*Am T*) noch gut erhalten. *Z*, Zahnscherbchen (Kontur verstärkt); *IR Bo*, Infrarostrale aus Bombinator-Knorpel.

B. Durchbrechende *normale* Zahnanlage im Zahnfeld des Spleniale einer Triton-Larve Gl. Stad. 45. *S*, Schmelz; *De*, Dentin; *Pr*, Praedentin; *Od*, Odontoblasten; *Sp*, Spleniale (Knochen); *Md*, Mandibulare (Knorpel).

C. *Reusenzahn* am Kiemenbogen (*K*) einer Triton-Larve Gl. Stad. 53. *Ep*, Epithelhülle ('Schmelzorgan'); *Z*, Zwischenschicht; *Od*, Odontoblasten; *GL*, Grenzlamelle des Epithels.

D. Die 4 Kiemenbogen (K1–K4) einer Triton-Larve Gl. Stad. 53 (Kopfquerschnitt). *Z*, Reusenzähnchen; *Si*, Sinnesknospen. (Konturen z. T. verstärkt).

(Manuscript received 14 : x : 54)



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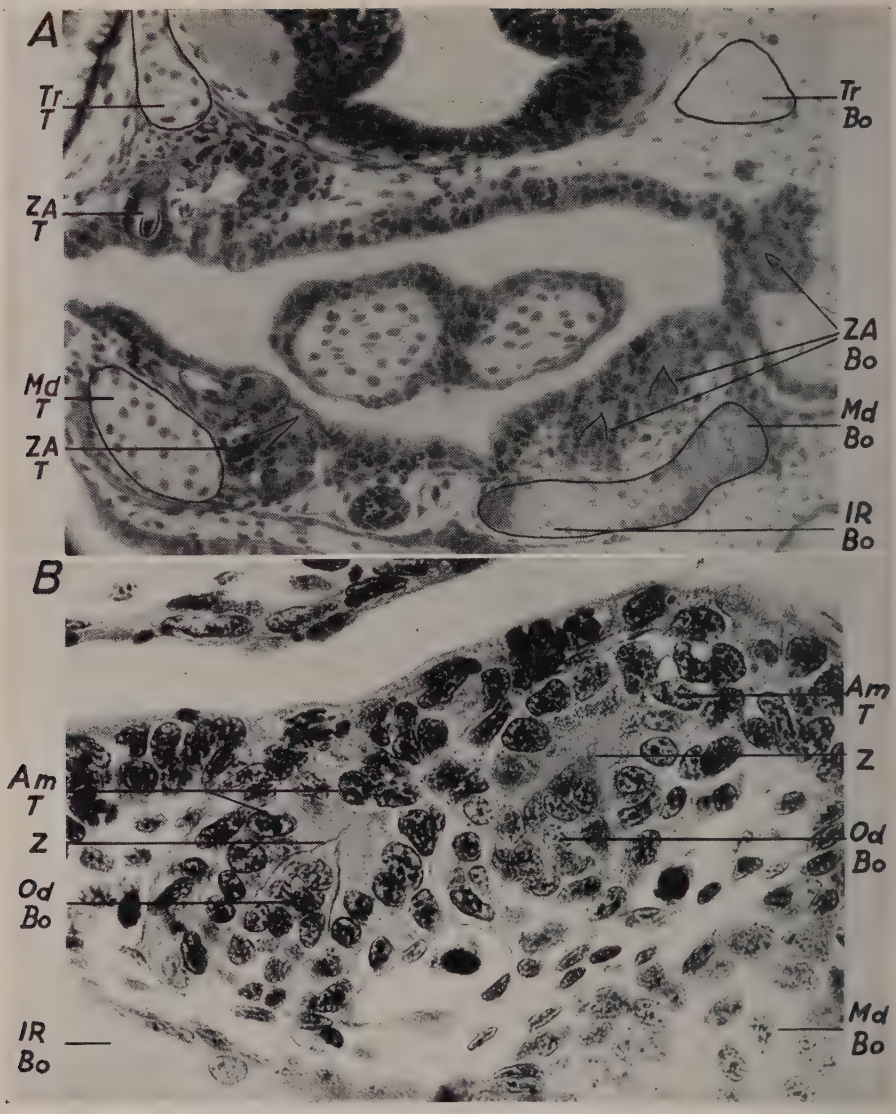
Tafel 1



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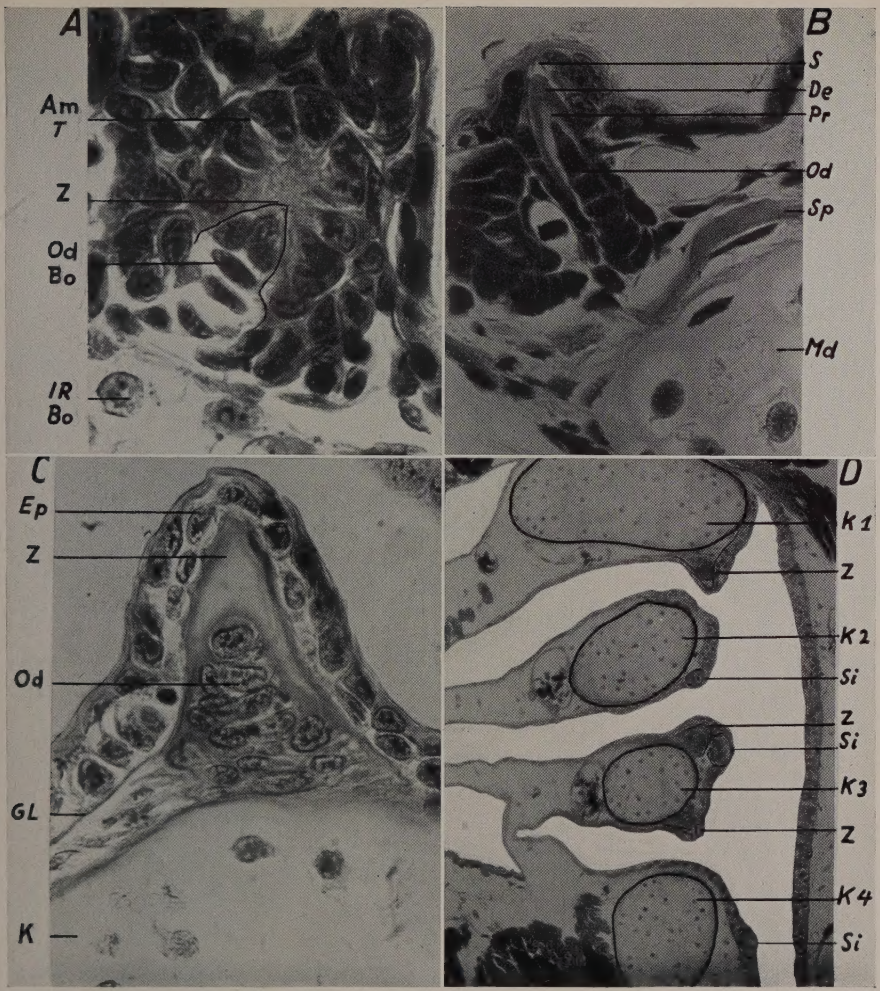

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Tafel 3

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